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Chemical Science and Technology Laboratory

1999 Technical Activities Report

Submitted to the Panel for the Chemical Science and Technology Laboratory Board on Assessment of NIST Programs National Research Council

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Chemical Science and Technology Laboratory
National Institute of Standards and Technology

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FEBRUARY 2000

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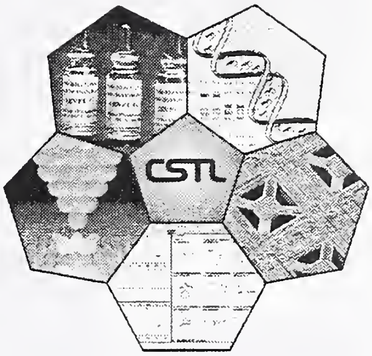


Program Overview

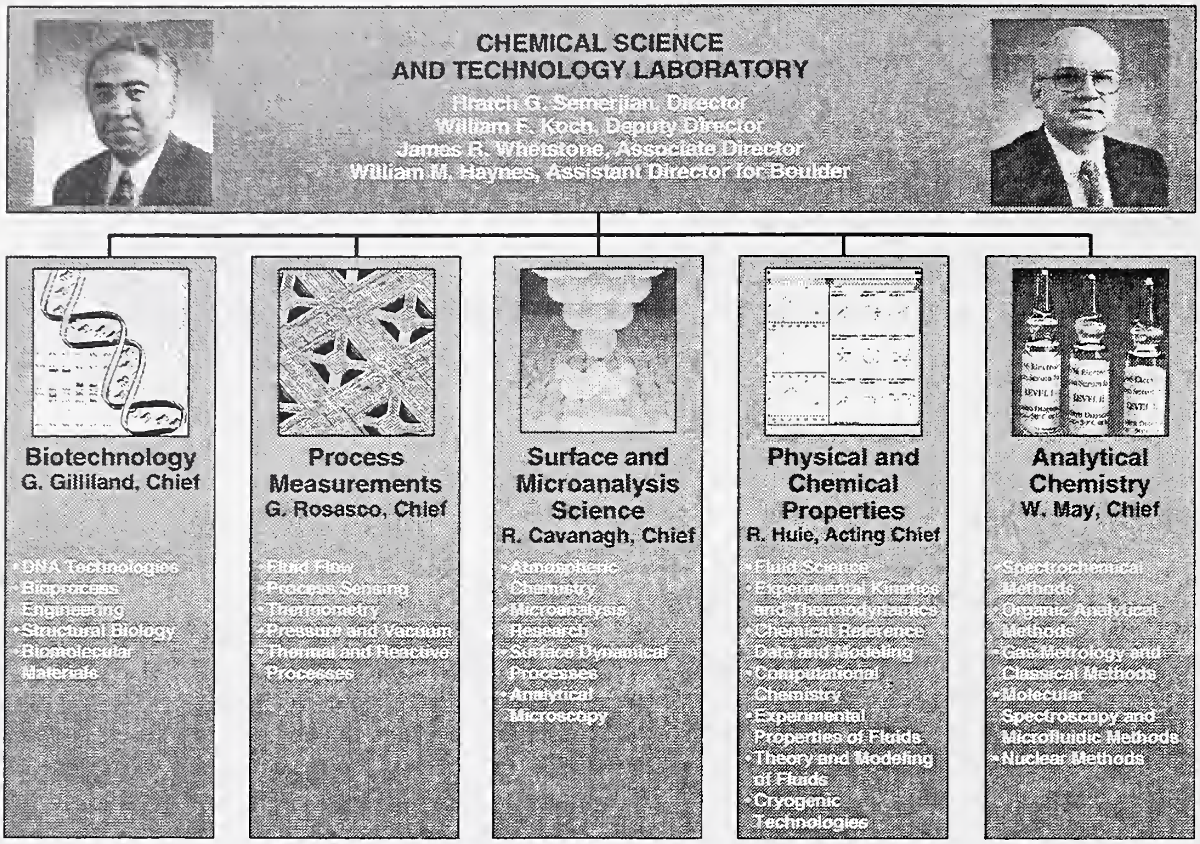
NIST, then the National Bureau of Standards (NBS), was created by Congress in 1901 as the leading source of measurements and standards provided to industry, commerce, scientific institutions, and all branches of government. Today, NIST is an agency of the U.S. Department of Commerce's Technology Administration. The **mission of NIST** is to strengthen the U.S. economy and improve the quality of life by working with industry to develop and apply technology, measurements and standards. It carries out this mission through four major programs: the Measurement and Standards Laboratories; the Advanced Technology Program; the Manufacturing Extension Partnership Program; and the Baldrige National Quality Program. Each program addresses different components of the science and technology enterprise.

Reflecting the importance of chemical measurements and standards to our country, the Chemistry Division was one of the first established by the founders of NBS. Today, the Chemical Science and Technology Laboratory (CSTL) has the most comprehensive array of chemical, physical, and engineering measurement capabilities of any organization working in chemical science and technology. CSTL, one of the seven NIST Measurement and Standards Laboratories, is an integral part of the NIST organization contributing to accomplishing the **NIST vision** to provide U.S. industry with the world's best technical infrastructure to promote economic growth and competitiveness.

Mission:

As the Nation's Reference Measurement Laboratory for chemical, biomolecular, and chemical engineering, the **mission of CSTL** is to provide the chemical measurement infrastructure to enhance U.S. industry's productivity and competitiveness, assure equity in trade, and improve public health, safety, and environmental quality. CSTL is a world-class research laboratory recognized by the nation as the primary resource for engineering measurements, data, models, and reference standards required to enhance U.S. industrial competitiveness in the world market. These capabilities are provided for the nation – U.S. industry, government agencies, and the scientific community. CSTL seeks to accomplish its mission by setting and meeting the following goals:

- ***Measurement Standards:*** Establish CSTL as the pinnacle of the national traceability and international comparability structure for measurements in chemistry, chemical engineering, and biotechnology, and provide the fundamental basis of the nation's measurement system.
- ***Chemical and Process Information:*** Assure that U.S. industry has access to accurate and reliable data and predictive models to determine the chemical and physical properties of materials and processes.
- ***Measurement Science:*** Address current, and anticipate next-generation, measurement needs of the nation by performing cutting-edge research in measurement science.

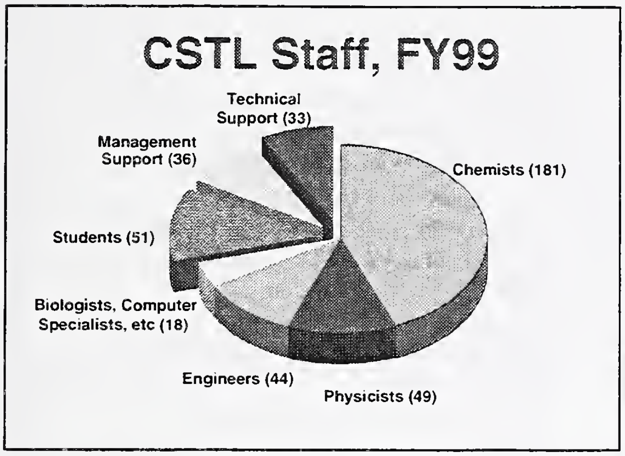


Organizational Structure:

CSTL is a multifaceted, synergistic organization with a broad customer base. The Laboratory has technical capabilities in analytical chemistry, surface chemistry and microanalysis, chemical and physical properties, process measurements and modeling, and biotechnology. The primary customers we serve are: industry (chemical, electronics, automotive, petroleum refining, instrumentation, biotechnology, environmental technologies, health care, etc.); federal, state, and local government agencies; standards and industrial trade organizations; and the academic and scientific communities. CSTL is organized to reflect the technical expertise that is the foundation of our technical program and allows us to accomplish our mission. The Laboratory consists of five Divisions: Biotechnology Division, Process Measurements Division, Surface and Microanalysis Science Division, Physical and Chemical Properties Division, and Analytical Chemistry Division.

Each Division employs a group structure organized to achieve synergy and critical mass in its technical program areas. To accomplish its mission CSTL maintains expertise in a broad range of technical areas. Our technical staff consists of chemists, physicists, engineers, biologists, and computer specialists located at the major NIST sites in Gaithersburg, Maryland and Boulder, Colorado, as well as at the Center for Advanced Research in Biotechnology (CARB) in Rockville, Maryland and the Marine Environmental Health Research Laboratory (MEHRL) in Charleston, South Carolina. The Physical and Chemical Properties Division has operations located at the NIST sites in Gaithersburg and Boulder. Biotechnology Division staff works closely with that of the University of Maryland Biotechnology Institute located at CARB. The Analytical Chemistry Division is the primary liaison with MEHRL, having staff located in Charleston. MEHRL is a cooperative research facility involving NIST, the National Oceanic and Atmospheric Administration, the South Carolina Department of Natural Resources, the University of Charleston, and the Medical University of South Carolina.

To achieve its goals, CSTL maintains an experienced, well-educated professional staff. The permanent staff numbered 290 in FY99, in addition to 122 temporary and part-time employees. Also, there were almost an equivalent number (234) of guest researchers who work closely with CSTL staff in various aspects of the research program. The technical capabilities of CSTL staff are extensive. CSTL technical staff hold degrees in chemistry (181), physics (49), engineering (44), and in biology and computer science (18). A technical support staff of 33 augments the professional staff. Approximately 75% (224) of the technical staff hold doctoral degrees.


CSTL Staff, FY99

The needs of our customers are reflected significantly in our program priorities. Customers purchase our products and services, *e.g.*, SRMs, Standard Reference Data, and calibration services, collaborate with us through consortia and CRADAs, contract for our services, and interact through round robins, conferences, and workshops, and informally with our technical staff. Some of the CSTL outputs and interactions in FY99 are summarized in the following table. Our research program results in new measurement methods, critical information for design of new or improved chemical or biochemical processes, and analytical methods or procedures supporting new SRMs.

Selected CSTL Outputs – FY 99

Div.	Pubs.	Talks	Committees[1]	Seminars	Conferences	CRADAs	Patents Issued	SRMs /RMs	SRDs	Cals.[2]
830	4	20	42	13	2	0	0	0	0	0
831	118	169	58	20	13	6	0	2	0	0
836	88	122	109	11	4	15	1	4	0	545
837	91	131	79	10	6	1	0	7	3	0
838	186	86	103	33	2	8	4	0	8	2
839	111	255	137	11	18	5	1	242	1	363
Totals	598	783	528	98	45	34	6	255	12	910

[1] Committee totals include 53 editorships
[2] Calibrations were performed for over 350 customers

Division Key:

830	Laboratory Office
831	Biotechnology Division
836	Process Measurements Division
837	Surface and Microanalysis Science Division
838	Physical and Chemical Properties Division
839	Analytical Chemistry Division

SRM – Standard Reference Materials

SRD – Standard Reference Data

Research Program Assessment and Evolution:

We balance our programs among development of essential measurement standards and technologies and basic research in measurement science to ensure a healthy science and infrastructural technology base for the future. Each Division in CSTL performs basic and applied research and maintains close contacts with the appropriate industrial and scientific communities to assess and anticipate the future measurement needs of our customer base. As part of our strategic planning process, in the fourth quarter of each fiscal year, we assess and prioritize future needs and re-orient our research program to reflect the changing needs of our customer base. This re-orientation is accomplished with existing resources. Areas are identified where new scientific or technological emphasis is required to meet emerging U.S. industry needs, or areas where the industrial need for products of existing programs is anticipated to increase significantly. Resources are redirected from activities where efforts have reached maturity and produced their anticipated results.

Program areas where CSTL has re-oriented its program over the last several years are shown at the right. Accomplishments resulting from these efforts are discussed briefly in this overview and in more detail in the Division reports that follow.

CSTL Base Budget Reprogramming
New Program Activities FY 97 – FY 2000

FY 97

- National traceability and international comparability for physical measurements
- Molecular scale materials characterization
- Computational chemistry
- Health care measurements
- International comparability of chemical measurements
- Bioinformatics

FY 98

- Standards for Raman spectroscopy
- Chemical analysis at critical surfaces and interfaces
- Non-metals in advanced materials
- Thermophysical properties of industrially-important chemicals
- Chemical analysis at critical surfaces and interfaces
- Computational chemistry,

FY 99

- Measurement methods and standards for next-generation health status markers
- New flow traceability paradigm
- Characterization of ultra-thin films
- Fluorescence intensity standards for flow cytometry
- Kinetics/thermodynamic data for industrial processes

FY 2000

- Tissue engineering
- Improved vacuum transfer standards
- Combinatorial techniques
- Cluster secondary ion mass spectrometry
- *In vitro* diagnostic device standards
- Fluid property databases for the chemical industry

Major Facilities:

Major progress has been made with facility additions affecting CSTL operations in FY99. The Advanced Chemical Sciences Laboratory (ACSL) on the NIST Gaithersburg Campus was completed in late 1998. Following approximately three months of preparation, the Analytical Chemistry and Biotechnology Divisions moved into the ACSL between February and May, 1999. The ACSL provides CSTL with 7,605 m^2 ($\approx$82,000 ft^2) of state-of-the-art laboratories. Although ACSL adds significantly to CSTL's research capabilities, it does not meet all facility needs of our program. Remaining is the need for laboratories with low vibration and high temperature stability necessary to push the state of the art in selected measurement science and standards areas. In the last few years NIST has developed a facilities master plan that envisions an additional facility, the Advanced Measurements Laboratory (AML), specified to meet low vibration/high temperature stability needs. Construction of the AML is expected to begin in FY 2000, with completion planned for 2004.

Technical Achievements:

Many noteworthy technical achievements were made by the CSTL scientific staff during FY99 that contributes to long term accomplishment of CSTL goals. Examples of these are discussed briefly below. More detailed information about these activities can be found in the overview and technical reports of the appropriate Divisions.

Measurement Standards

Development and dissemination of national measurement standards is a core NIST mission. NIST disseminates units of measure to users in the U.S. through vertical traceability structures that provide U.S. industry with the measurement infrastructure necessary to maintain and advance economic growth. CSTL is responsible for two SI base units, temperature and the amount of substance (mole), in addition to the derived units of pressure, vacuum, gaseous leak rate, humidity, fluid flow rate, liquid volume and density, and air speed. National measurement standards for these quantities are developed and disseminated by CSTL and are compared with those of other nations. The latter effort has become increasingly important in the decade of the 90's as the impact of international trade on the U.S. economy has grown.

Traceability to national measurement standards, *i.e.*, vertical traceability, provides U.S. industry with access to primary measurement standards and methods disseminated by NIST. Instrument calibration and related test services and SRMs are the primary means used to disseminate U.S. national standards to industrial users and federal, state, and local government laboratories. Development and dissemination of SRMs is the primary method to disseminate chemical measurement standards, although SRMs are also used to disseminate some physical measurement standards. During FY99 CSTL provided 53 % of the SRM units sold through the NIST Standard Reference Material Program and 17 % of the calibration services provided through NIST's Office of Measurement Services

Vertical Traceabilty – Physical Measurements:

The Process Measurements Division of CSTL is responsible for realizing, maintaining, and disseminating national measurement standards for temperature (in the range of 0.65 K to 1235 K), pressure, vacuum, gaseous leak rate, humidity, fluid flow rate, liquid volume and density, and air speed. (More detailed descriptions of these activities are contained in the Process Measurement Division's report.) Customer-appropriate access to national measurement standards requires CSTL to provide a range of services and activities:

- maintenance and improvement of primary standards,
- performance of instrument calibrations and tests, operation of Measurement Assurance Programs,
- proficiency and round-robin tests, development of mechanisms for realization of secondary standards in customer laboratories, and
- consultation and customer assistance services.

In FY99 a web-based information management system was implemented that allows us to monitor progress and assess the performance of our calibration services. Customers can now access status information on instruments that they have submitted for calibration. In a recent study, we found that performance was generally good and quality high, although turnaround time could be improved. Attention to these issues has resulted in significantly improved performance and service to our calibration customers.

Vertical Traceabilty – Chemical Measurements:

CSTL's Analytical Chemistry Division continues to fulfill the NIST mission to define, establish, and broaden the national infrastructure for chemical measurements by providing the national standards for the SI base unit of the amount of substance (mole). Currently, NIST provides nearly 1300 different types of SRMs to customers. In FY99 approximately 33,000 SRM units were sold to about 6,550 unique customers; over 17,000 units (53%) of these represent the 850 different SRMs certified for chemical composition. Provision of SRMs broadens the U.S. chemical measurement infrastructure by providing traceability of chemical measurements to national standards. They are also the foundation for the considerable emphasis that has been placed on the assessment of the comparability of chemical measurement standards in the international arena for several years.

SRMs are the primary means of disseminating chemical measurement standards, their development and production is a primary focus of CSTL efforts which were focussed in four areas in FY99:

- nutritional labeling for food
- health-care,
- the environment, and
- general industry needs.

International Comparisons of U.S. National Measurement Standards:

Assessment and recognition of the comparability of measurements and standards among nations are obligations derived from the Treaty of the Meter and are the responsibility of National Metrology Institutions (NMIs). NIST is the U.S. NMI and is solely responsible for comparison of U.S. national measurement standards with those of our trading partners. The impact of measurements and standards issues have increased in importance because they have become closely linked to trade issues during the decade of the 90's as global trade has become a central element in U.S. economic growth. The substantial dismantling of tariff-based trade barriers has enhanced world trade. However, the opportunity to erect technical trade barriers based on product conformity testing has assumed an increasingly important role in limiting or excluding access to global markets. International documentary standards, and the product conformity testing requirements that are supported by them, are highly measurement intensive and can provide an effective means to exclude U.S. exports from foreign markets. Demonstrating compliance with product conformity specifications has become an increasingly important requirement for entry of U.S. products into much of the global marketplace. Conformity demonstration is based on measurements that are often required to be traceable to the *importing nation's national measurement standards* or to standards recognized as such. Therefore, recognition that measurements traceable to U.S. national measurement standards are equivalent to those of our trading partners can be a critical issue for access of U.S. products to global markets. The International Committee on Weights and Measures (CIPM), established under the Treaty of the Meter at the turn of this century, the NMIs, and the regional metrology organizations (RMOs) have become focal points in the drive to demonstrate levels of comparability and equivalence of national measurement standards.

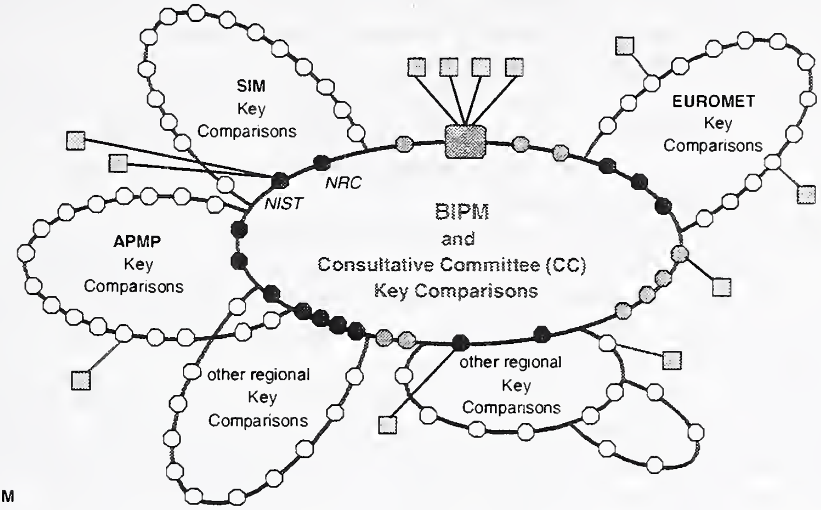
CIPM is charged with developing international comparisons of primary measurement standards among NMIs and those maintained by the International Bureau of Weights and Measures (BIPM). It uses its Consultative Committees (CCs) to organize comparison efforts. Each CC is responsible for a SI unit and related quantities. Comparison efforts involving the more industrialized signatories to the Treaty of the Meter have been conducted for decades. Greatly increased demand for participation in comparison efforts has been the result of the growth in trade and the fall of tariff-based trade barriers in the last two decades. RMOs have been formed in the last two decades to deal with the increased importance of trade and the comparison of national measurement standards among nations within various geographic regions. These organizations include SIM (Systema Interamericano de Metrologia – comprising North and South America), APMP (the Asian-Pacific Metrology Program), and EUROMET (the Metrology Organization of the European Union). Planning activities and working relationships have been established among regional metrology organizations and the CIPM to develop a global system of comparability assessment that supports recognition of measurements and standards globally (see Figure on succeeding page).

NIST has established a leadership role in these efforts and CSTL is involved significantly in efforts to develop comparison assessment strategies, methods to report and compare results, and compare both chemical and physical measurement standards. These efforts have been pursued aggressively by the two CSTL Divisions, Analytical Chemistry and Process Measurements, having major responsibilities in this arena.

International Comparisons – Physical Measurements:

NIST remains the only NMI in the world that has successfully realized the International Temperature Scale of 1990 (ITS-90) over its full range for contact thermometry (0.65 K – 1235 K). Having been the first NMI to complete realization of the scale, increased emphasis in the Process Measurements Division has been given to comparison of temperature standards with those maintained by other NMIs. Several comparisons of realizations of the ITS-90 over the range of the scale at the highest accuracy levels are in progress. Central to this effort are the Key Comparisons (KCs) organized by the CIPM Consultative Committee for Temperature (CCT). CSTL participated in four KCs in FY99 that are in various stages. In one case, KC 3 [83.8058 K (argon triple point) to 933.473 K (aluminum freezing point)], we fulfilled responsibilities as the pilot laboratory (organization and data reduction), which involved 14 NMIs. In addition comparison efforts within SIM have either been planned or started.

Framework for International Comparability Assessment



▨ BIPM

◍ NMI participating in BIPM/CC Key Comparisons

● NMI participating in BIPM/CC Key Comparisons and in regional Key Comparisions

○ NMI participating in regional Key Comparisons

☐ NMI participating in neither BIPM/CC nor regional Key Comparisons but making bilateral comparisons directly with BIPM or with NMIs in categories ◍ ● or ○

BIPM = Bureau International des Poids et Mesures
CCQM = BIPM Consultative Committee on Amount of Substance
RMOs = Regional Metrology Organizations
NMI = National Metrology Institution
SIM = Systema Interamericano Metrologia
EUROMET = Metrology Organization of the European Union
APMP = Asian-Pacific Metrology Program

As with temperature standards, international comparisons of **pressure and vacuum** standards has grown in magnitude with CSTL participating in six key comparisons. These are organized under the CIPM Consultative Committee for Mass and Related Quantities (CCM). NIST is serving as the pilot laboratory for three of these comparisons that require development of total new transfer standards, instrumentation, and protocols. Two sub-atmospheric comparisons were formally completed in FY99, with data analysis and a draft report for participant circulation to be completed in 2000. Circulation of the transfer standard for the vacuum comparisons began in the spring of FY99, with completion scheduled for the fall of 2000. With the exception of the vacuum comparison, NIST has now completed testing in all the key comparisons. Comparison effort involving member NMIs of SIM are also underway.

In the areas of fluid transfer and air speed measurement standards, several efforts have been started or are in progress. NIST joined 11 EUROMET laboratories to compare **air speed standards** over the range 0.3 m/s to 20 m/s. A comparison **of liquid volume standards** has been conducted over the range from 50 milliliters to 50 liters involving the NMIs of Canada and Mexico. The comparison at the 50 liter level also included the NMI of Germany, PTB. Comparisons of **liquid flow rate** measurement standards using 200 mm tandem orifice meters have been completed. For these comparisons, domestic and industrial testing laboratories as well as NMIs participated. NIST's results were within stated uncertainties for all participants. A new and important aspect of this effort was the use of real-time monitoring of flow profiles during the testing. This capability was developed by CSTL and enables measurement of the velocity profiles entering the metering package providing a means to achieve greater consistency in performance of the flow instrumentation used.

These efforts, coupled with those in chemical measurements, place NIST in a leadership role in the NMI community. The necessary level of effort is significant and is expected to continue for several years. The capability to effectively meet the challenge represented by increased emphasis and importance of international trade issues, is in part due to prior year investments made in these areas through CSTL base budget reprogramming.

International Comparisons – Chemical Measurements:

From the perspective of SI unit classification, all chemical measurement standards are measures of the amount of substance, the mole. The CIPM Consultative Committee on Amount of Substance (CCQM) addresses issues of comparability and traceability of measurement standards. The CCQM has begun to assess chemical measurement comparability through strategically selected comparisons among national chemical metrology laboratories in the general areas of advanced materials, biotechnology, commodities, the environment, food, forensics, health, pharmaceuticals, and general analytical applications. NIST and CSTL play a prominent role in these efforts. The Analytical Chemistry Division is a world leader in chemical metrology and is leveraging that expertise to influence the agenda of international comparisons in chemical measurements. Fifteen international comparisons have been conducted and CSTL has participated in all of them. The CCQM has formed five working groups:

- gas analysis,
- organic analysis,
- inorganic analysis,
- pH and conductivity, and
- key comparisons.

CSTL provides leadership and critical participation in all of these activities

CSTL is also very active in SIM and holds the Chair for the Chemical Metrology Working Group within the Interamerican System of Metrology (SIM). Since only 3 of the 34 countries have formal programs in chemical metrology, we conducted five training courses in FY99 in the areas of organic, spectrochemical, nuclear, gas metrology and classical analytical methods. The number of participants ranged from 6 to 15 in these one-week, hands-on courses given for current or designated future leaders of chemical metrology programs of NMIs within SIM. Six comparison exercises have been developed to test the proficiency of SIM NMIs.

The NORAMET sub-region of SIM consists of NIST, NRC-Canada, and CENAM-Mexico. All produce Certified Reference Materials (CRMs) and have agreed to compare their capabilities while assisting in each other's reference materials certification campaigns. Comparisons for metals in drinking water, elements in sediments and mussel tissue, organic compounds in sediments and fish tissue, methyl mercury in fish tissue, and vitamins and minerals in milk powder took place in FY99 using this approach.

For six years CSTL been involved in a strategic bilateral program with the National Measurement Institute (NMi) of The Netherlands for determining the equivalence of primary gas standards. These efforts have resulted in a formal "Declaration of Equivalence" that is mutually recognized by the U.S. EPA and European environmental regulatory agencies as documenting the equivalence of eight NIST and NMi primary gas mixture suites (spanning a wide range of concentrations). In FY99 equivalence in natural gas standards have been established; efforts have continued to address a bias of 2% observed between NIST and NMi primary standards for hydrogen sulfide. Additional collaborations for assessing the equivalence of primary standards have been established with DFM (Denmark) and OHM (Hungary) for conductivity, with the PTB for pH, with EMPA (Switzerland) for elemental solution standards, with NIMC for volatile organic compound standards, with INPL (Israel) for moisture in oils, and with NPL (UK) for optical absorbance filter standards.

More detailed discussions of these efforts can be found in the reports of the Process Measurements and Analytical Chemistry Divisions.

International Comparisons Database:

The NMIs, and the International Bureau of Weights and Measures are developing a frame-work for establishing mutual recognition of measurements and national measurement standards. Such recognition will be based on measurement and calibration capabilities that are supported by measurement comparison data. The implementation of an International Comparisons Database (ICDB) was one of the key action items in the recently agreed upon CIPM Mutual Recognition Arrangement (CIPM MRA), a framework signed by the National Metrology Institutes (NMIs) of 38 nations. The MRA is designed to help resolve trade disputes that arise over differences in measurements and standards. In anticipation of the MRA, a CSTL-led team began designing the ICDB in early 1998. The BIPM joined the effort in 1999, providing the data for over 240 key and supplementary comparisons. The ICDB was launched simultaneously on web sites at NIST and the BIPM on November 30, 1999.

The MRA establishes a formal system of "key" measurement comparisons among the NMIs in the signer nations. These comparisons establish how closely a particular measurement, *e.g.*, voltage, force or length, performed at one NMI agrees with results of counterpart institutes in other countries. Levels of agreement establish the basis for linking measurements across international borders.

Initially, the new database contains information on the design protocols and the participants of completed and ongoing key comparisons among the NMIs of nations that signed MRA. About 130 of these round-robin measurement exercises are now under way. Once the Consultative Committees of the CIPM evaluate the reliability of the actual measurement results, they will be cleared for entry into the database. Once entered, results between pairs of participants or between any participant and a reference value can be directly compared.

The database will also contain lists of calibration and measurement service capabilities asserted by each participating NMI. The CCs will analyze the results of the key comparisons and examine whether the results are sufficient to support the levels of accuracy reported by an NMI for a particular measurement service.

CSTL will also lead the NIST effort to implement the database within the SIM. Comparison data from the SIM and the other Regional Metrology Organizations will be entered into the database to link regional comparison data with the CIPM efforts. Furthermore, NIST will begin to implement the database to organize calibration data within the U.S. system of measurement traceability. The ultimate goal is to provide a recognized and readily available means to compare measurement data anywhere along the chain of national systems of traceability and international comparability.

Measurement Standards – Technical Highlights:

A tool that is finding increased application in industrial process control and quality assurance is **Raman spectroscopy**. Intensity calibrations are essential to make Raman-based measurements reliable and instrument independent. CSTL is addressing the need for reliable and accurate intensity standards using three different glasses that were identified for three different wavelength regions. These glasses were shown to be homogeneous in composition and resistant to photobleaching, even under laser irradiation. The Raman intensity from this irradiation is being calibrated against radiometric white light sources. When this characterization is complete, these glasses will be used in a round-robin measurement comparison through ASTM E13.08 to determine intensity-corrected Raman spectra.

To provide standards that support **improved quality control in DNA sequencing**, a human mitochondrial DNA (mtDNA) (SRM 2392) has now been sequenced for applications ranging from forensics to the characterization of disease-specific mutations. The effects of incorrect sequencing can have broad impact. For example, a false positive might lead to the conviction of an innocent individual, while a false negative might lead to manifestation of a disease that might otherwise have been prevented. This SRM will help investigators who perform polymerase chain reaction amplification and sequence human DNA to assure the accuracy of sequence information. An inter-laboratory evaluation has been completed. SRM 2392 is now available for purchase. Corroboration of sequence information with that of the SRM provides quality assurance in sequencing procedures and the associated forensic identification or medical diagnosis.

The growing concern about the potentially adverse health effects of **particulate matter with an aerodynamic diameter <2.5 µm** ($PM_{2.5}$) has created a demand for new standard reference materials. One such standard is SRM 2784, a filter loaded with SRM 1649a, Baltimore Dust. This dust is an urban $PM_{2.5}$ dust, containing many of the constituents inhaled by city dwellers. This dust has been carefully loaded onto filters. Visual inspections indicated an even distribution of this dust throughout the filter. A carbon analysis will provide further assurance of within-filter homogeneity, and x-ray fluorescence measurements will determine how uniform the elemental constituents are across the filter. These carefully characterized filters should prove invaluable to labs that test and characterize filters taken from the field. Because of the high demand for this filter, "prototype" filters have been released as a reference before certification as an SRM. Having recognized the importance of particulate matter filter SRMs, the Environmental Protection Agency is expect to help defray the cost for production and certification of SRM 2784.

Quantitative measurement of **black carbon,** also known as elemental carbon or soot, is a challenging measurement of long standing. Black carbon is a tracer of fire, a component of particulate air pollution, and has been implicated in adverse effects on human health and global climate change. Its accurate measurement has been beset by two problems: first, black carbon is not a single chemical entity, and second it is usually present at very low levels. To address the first problem an international comparison was arranged, involving a multidisciplinary team using a broad range of chemical, thermal, and optical methods to measure black carbon content in an appropriate reference sample, NIST SRM 1649a (Urban Dust). The results have been evaluated and will be incorporated as informational values in the new Certificate of Analysis for the SRM. This is the first effort to provide method-specific black carbon reference values. The second problem has been approached by developing a new method for black carbon analysis. This method, the thermal optical kinetic technique, uses ultrahigh purity gases, a quartz oven, and measures spectral absorbance and carbon reaction rate as the means to sense the onset of black carbon oxidation. The reaction can then be stopped, and the minute traces of black carbon residues may be isolated and subjected to further tests. The sensitivity of the method makes it an excellent candidate for quantitative apportionment of atmospheric carbon.

Chemical and Process Information

Realistic design and modeling of chemical processing systems is critically dependent upon chemical, physical and engineering data to accurately describe materials properties and processes and to model the mechanisms controlling these systems. CSTL activities provide or contribute to:

- standard/evaluated benchmark data for properties of important substances, classes of substances and systems,
- data collections, data prediction methods, and models to meet high priority/immediate industrial and/or national needs,
- development of consensus standards for key properties, substances, and processes, and
- protocols or procedures for data access, facilitation of data exchange, and dissemination of appropriately formatted data.

The increased recognition that structure is important to understanding biological function has fueled a demand for an authoritative, comprehensive, and current source of structural data. Responsibility for **the Protein Data Base (PDB)**, a NSF/DOE/NIH funded effort, was moved to the **Research Collaboratory for Structural Bioinformatics (RCSB)** in FY99. The RCSB is composed of groups from Rutgers University, the University of California at San Diego Supercomputing Center, and the CSTL Biotechnology Division. The PDB is the single most comprehensive international repository in the world for the processing and distribution of three-dimensional structure data of biological macromolecules. The users of this database can enter structures determined experimentally by NMR, X-ray crystallography, and other biophysical methods. These structures are then annotated and validated by members of the Collabortory. Currently, up to seventy-five structures are entered per week, and the web-site has more than a million hits per month. The PDBs approach of using modern data management practices should prove an invaluable resource for structural genomics initiatives worldwide.

CSTL organized the working group Annex 18 of the International Energy Agency in the early 1990's as a means of developing and promulgating standards for **alternative refrigerants**. In 1999 Annex 18 concluded its third and final phase by completing comprehensive evaluations of the available equations of state and sanctioning standards for R123, R134a, R32, R125, and R143a. Wide participation was invited in this process, resulted international recognition of this work. Of the five fluids, the formulations for R123 and R143a developed at NIST were designated as international standards. The final report for Annex 18 was presented at the quadrennial Congress of the International Institute of Refrigeration. Additionally, the ISO has recently approved the establishment of a working group to develop standards for refrigerant properties. CSTL will be active in this group, along with many of the Annex 18 participants.

Industrial combustion systems, such as power generation plants, increasingly rely on **fluid dynamics simulations to provide real-time control** of the combustion byproducts and process efficiency. Benchmark data are needed to validate the sophisticated models underlying these calculations if such simulations are to be accurate predictors and, thereby, become widely accepted. Extensive amounts of data have been collected at the NIST reference-spray combustion facility in support of this objective. To make this data available to a wide audience, this year a preliminary version of this database was released to industrial and academic collaborators for their use in validating fluid dynamics models. All potential collaborators were invited to a workshop to facilitate use of this database. The objectives of this workshop were to familiarize the participant with the NIST combustion facility, to present recent results from this facility and to assess future measurement needs. Discussion focused on the most critical industrial needs and how the NIST reference spray combustion facility can provide data with the highest impact.

A new effort to develop chemical kinetic data for supercritical water (SCW) reactions began in FY99 with the completion of an experimental apparatus that will provide the basis for these measurements. The reactor is designed to operate well above the critical point and has precise temperature and pressure controls. Reactants can be injected directly into the SCW reaction environment. Micro-liter samples can be obtained with minimal disturbance of the ~124 mL reaction environment. Initial results have been obtained in the study of the reversible dehydration of alcohols. These experimental capabilities are the basis for studies supporting a detailed understanding of the chemical reactions of organic compounds in SCW processes. Data of this type will be the basis for process development in the areas of hazardous waste destruction, oxidation of biomass and metabolic wastes, and development of new chemical synthesis strategies.

Data analysis tools are developed in the Surface Science and Microanalysis Division to advance the broad and meaningful application of micro-analytical methods to significant areas of interest. One example of this role has been in the development and utilization of the NIST **x-ray database and spectral analyzer program**. A striking, and historically interesting example of the power of this database and analyzer combination was demonstrated this year. Noddack *et al.* claimed to have discovered element 43 in 1925 and dubbed it masurium (Ma). This claim was widely discounted at the time, and the discovery of element 43 is usually attributed to Perrier and Segre's work of 1937, in which the element was named technetium (Tc). Using the experimental configuration deduced from the 1925 paper, DTSA was used to simulate the 1925 experiments for a range of sample compositions. The relative intensities of the various x-ray lines and the peak-to-background ratios were determined and compared to the x-ray spectrum that Noddack *et al.* published. The lines in the calculated x-ray spectrum and the background levels are consistent with the published spectrum for the sample composition used in the 1925 experiment. There appears to be no reason from their spectral information to dismiss the claim that Ida Noddack and coworkers discovered element 43.

The NIST WebBook (http://WebBook.nist.gov) continues to be a prime example of using the worldwide web for the effective dissemination of NIST reference data to a large number of customers. During FY99 the fifth edition was released. The total number of compounds for which data are provided has steadily increased and in the fifth edition, data for more than 31,800 compounds are available. As with every previous release, new data types were added. A new tool for substructure searching using chemical structures drawn by the user was also added. Between 6,000 and 12,000 users per week use the Chemistry WebBook, an increase of about 25% from last year. The fraction of returning users is between 45% to 55%. The WebBook has the potential to be a single point of entry to access all chemical data at NIST. It is anticipated that during FY 2000, there will be two releases of the NIST WebBook. Additional data as well as new searching software will be included.

Models that predict the behavior of advanced chemical processes occurring in a **plasma process reactor** not only explain how a particular reactor works, but can also be used to design better processes. An example of such an application was the demonstration of a new method for controlling the spatial distribution of reactive chemical species. Normally, radio-frequency power is applied to one of the plasma-generating electrodes and the other electrode is grounded. Simulations using a model developed at NIST predicted, however, that by varying the electrical loading of the second electrode, the flow of current through the plasma could be directed to the other electrode or to other surfaces of the reactor. This, in turn, controlled where the plasma electrons are heated and reactive species generated. This method was demonstrated in CF_4/O_2 and C_2F_6/O_2 plasmas. The spatial distribution of the CF_2 radical was measured using two-dimensional laser-induced fluorescence. Varying the load impedance was shown to give greater control over the spatial distribution of CF_2 than could be obtained by varying pressure, power, gas mixture, or flow rate. This control method could potentially be used to direct reactive species in chamber-cleaning plasmas toward the reactor surfaces most in need of cleaning or to increase the uniformity of reactive species across the wafer surface during etching.

Measurement Science

CSTL maintains a strong research program to support the Nation's measurements and standards infrastructure, establish new measurement capabilities supporting new or advanced technology development and dissemination, and provide a fundamental basis for global scientific decision making. There have been many noteworthy research achievements by CSTL scientists and engineers during FY99. A brief discussion is presented below. More detailed information is contained in the technical reports of the respective Divisions found in the remainder of this report.

Because low-level gaseous contaminants often cause deleterious effects in manufacturing processes, a need for sensors with improved sensitivity and accuracy has arisen. Conventional techniques for generating and monitoring low partial-pressures of active gases, such as water, are limited by adsorption and the inherent reactivity of the monitoring devices. Work in the Process Measurements Division has sought to solve this problem using a method referred to as **single-mode cavity ring-down spectroscopy**. This laser-based spectroscopy technique measures the decay time of a single longitudinal mode of an optical cavity to measure the concentration of a contaminant. Experiments demonstrated that the single mode approach was significantly more precise and stable than conventional implementations of this spectroscopic technique. Specifically, a measurement imprecision of ~0.3 % over many days was demonstrated. This level of precision should enable a new generation of standards for measurement of humidity (moisture concentration in gases) and of vacuum (partial pressures of gases).

Characterizing the structure of new polymeric materials is an important component of understanding and controlling the properties of these materials. **Near-field scanning microscopy at infrared wavelengths** is one of the emerging technologies for such nondestructive analyses. Researchers in the Surface and Microanalysis Science Division have developed a novel instrument to make such measurements. The key components of this system are an infrared laser with 150 cm^{-1} bandwidth, an infrared focal plane array, and a near field probe fabricated from fluoride glass fiber. Transmission spectra of patterned samples have demonstrated a spatial resolution of 350 nm. Absorption spectra of thin, polymeric films have demonstrated the ability to measure multi-wavelength spectra across the film. These types of multiplexed images will be used to identify sample inhomogeneity and study blend phase-segregation.

Advances in magnetic materials research rely increasingly on information about **chemical distributions at near-atomic dimensions**. One approach to obtaining this information is analytical electron microscopy, which is capable of both imaging and chemical analysis with high spatial resolution. This technique has been used to image samples of Sm:Co:C nanocomposites with a resolution better than 0.2 nm. The resulting data are used to create elemental maps of the composition, providing important clues as the magnetic properties of these materials at very fine length scales.

The biosynthetic pathway leading from **glucose to chorismate and its aromatic derivatives** has major potential to produce industrially significant aromatic compounds. The Biotechnology Division is using its broad expertise in site-directed mutagenesis, NMR spectroscopy, and X-ray crystallography and diffraction to determine and analyze molecular structures necessary to understand enzyme catalytic mechanisms, regulation, and biochemical and biophysical properties. Such knowledge is required to understand the reactions found along the chorismate pathway. Through collaborations with industry and government laboratories, enzymology and structural investigations of several key components that may be amenable to engineering for improved pathway throughput have been initiated. A database that combines existing structural and related information with new biothermodynamic measurements has been created. The chorismate metabolic pathway is being developed as a model system for bioprocess measurement and modeling technology.

In FY 99 an **advanced methodology for high precision chemical analysis** was demonstrated by Analytical Chemistry Division staff. This method achieves improved analytical chemistry laboratory performance using commercial instrumentation and innovative, advanced signal analysis methods. Specifically, inductively coupled plasma-optical emission spectrometry (ICP-OES) has been demonstrated with instrumental measurement precision of a few parts in 10,000 (0.02% - 0.2%). This capability for chemical sample analysis is competitive with more difficult and labor-intensive approaches, especially "classical chemical analysis" that is both labor intensive and expensive and a disappearing capability in analytical chemistry. However, for most laboratories, it is the only methodology available to achieve industry-required precision in the determination of the major and minor material components.

This high precision ICP-OES methodology is used to develop new NIST Traceable Reference Materials programs and related activities, *e.g.*, commercial production of NIST-traceable standards for instrumentation calibration used for inorganic analysis (spectrometric solutions). Protocols based on this new high-precision ICP-OES approach will be transferred to commercial standards producers to transfer the accuracy embedded in NIST SRMs to their products. This new technology will also be used at NIST for blind audits of NTRMs lots. In addition, this methodology is being used to support standards development for a new Department of Energy tritium production program ["Estimated to cost $400M over 15 years, billions less than alternative options."]. Although only a small portion of the program, DoE officials have stressed the need to transfer this NIST methodology to DoE contractors to achieve the stringent material specifications DoE has set. Broader application of the research (to other instrumental techniques) are being explored as an opportunity to promote technology transfer by working with analytical instrument manufacturers.

Hybrid-bilayer membranes are promising matrices new bioprocesses, such as the development of pharmaceutical. However, **the bilayer formation mechanism** can affect the composition of the membrane and hence its function. To understand how differing growth conditions change these membranes, a noninvasive probe is required. One such probe is sum frequency generation, a nonlinear optical process that exploits the asymmetry found at interfaces. In sum frequency generation, two lasers operating at different wavelengths are "summed" together to give light at a third wavelength. Because an asymmetry is required for this process, only the interface regions are probed. To demonstrate that this approach could be used to monitor hybrid-bilayer membrane formation, CSTL scientists in the Biotechnology and the Surface and Microanalysis Science Divisions used a unique, NIST-designed laser system to monitor the fusion of d13-dipalitoyl-phosphatidylcholine vesicles. The magnitude of the sum frequency signal grew in time, as vesicles fused. Moreover, by determining which wavelengths the sum frequency generation process was most efficient, it was possible to determine what functional groups reside at the vesicle interface. These results demonstrate that sum frequency generation can be used to study molecules for which the structure and properties are likely to be important to the function of hybrid-bilayer membrane-based processes.

There is an increased appreciation for the fact that biological activity is sometimes greatest for molecules in improbable structural configurations. Identifying and understanding which configurations are active has potentially wide-ranging biochemical significance. To explore this further, CSTL scientists have built a confocal fluorescence microscope that combines **single-molecule fluorescence spectroscopy with single ion-channel electrophisiology**. Experiments during this past year investigated small-molecule diffusion in a planar, lipid bilayer. Among the surprising discoveries was optical trapping occurs at a laser fluence 100 times lower than predicted theoretically. The electrical conduction of bilayers is under investigation using electrical current recordings of individual channels that reveals two discrete sets of molecules, each with different electrical conductance properties. Understanding function and reactivity on a molecular scale, as this technique allows, has exciting implications for future design and improvements of biocatalysis and other bioprocesses.

Awards and Recognition:

Many CSTL scientists and engineers received awards and recognition of their work in FY99.

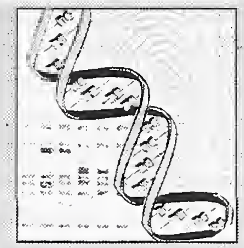
- **Charles M. Beck** was the recipient of the ASTM Lundell-Bright Award.
- **Pedro I. Espina** received the "Best Paper" award at the 1999 Measurement Science Conference.
- **Robert Fletcher** received the 1999 Sigma Xi award for outstanding service to the NIST chapter.
- **Joseph T. Hodges, J. Patrick Looney, and Roger D. van Zee** received the Department of Commerce Bronze Medal for their research that produced fundamental advances in the theory and practice of Cavity Ring-Down Spectroscopy.
- **Richard F. Kayser** was awarded the Department of Commerce's Silver Medal in the leadership category in recognition of his role in establishing a superb technical program with direct industrial impact while he served in Physical and Chemical Properties Division.
- **Michael J. Kurylo** received a Certificate of Appreciation from the United Nations Environmental Programme.
- **Eric W. Lemmon, Mark O. McLinden,** and Adele P. Peskin (895) received an SRD Measurement Service Award for their contributions to the development of the REFPROP Database.
- **Joseph W. Magee** was given the Diversity Award for the NIST-Boulder Laboratories.
- **Eric D. Marquardt and Ray Radebaugh** received the Russell B. Scott Memorial Award for the Outstanding Paper in Cryogenic Engineering Research presented at the Cryogenic Engineering Conference.
- **Mark O. McLinden** received the NIST Slichter Award for working closely with the air-conditioning/refrigeration industries to replace ozone-depleting CFCs with environmentally acceptable alternatives.
- **Michael R. Moldover and Robert F. Berg** were part of a team which received the NASA Lewis Distinguished Publication Award.
- **Michael R. Moldover** was recognized for presenting the best oral presentation at the Seventh International Symposium on Temperature and Thermal Measurements in Science and Industry.
- **Dale Newbury** was co-recipient of the 1998 Best Paper award from Electronics and Electrical Engineering Laboratory at NIST.
- **Patrick A. G. O'Hare** (retired) was made a Fellow of IUPAC.
- **Ray Radebaugh** was awarded the J&E Hall Gold Medal from the Institute of Refrigeration for research on the development and theory of pulse tube refrigerators.
- **Gregory J. Rosasco** was awarded the Department of Commerce's Silver Medal in the leadership category in recognition of his role in establishing standards and services in the Process Measurements Division which meet industry's needs and are recognized for their outstanding quality worldwide.
- **Jan V. Sengers** has been elected a Fellow of the American Institute of Chemical Engineers.
- **Steven E. Stein** received the 1999 ANACHEM award from the Association of Analytical Chemists.
- **Steven E. Stein** was selected to receive the Patterson-Crane Award by the Columbus and Dayton Sections of the American Chemical Society.
- **James Stivers** of the Structural Biology Group was awarded the FY99 CSTL Technical Achievement award for his work on the kinetic mechanism of damage site recognition and uracil flipping by *Escherichia coli* uracil DNA glycosylase.

II. Biotechnology Division (831)

Gary L. Gilliland, Chief

A. Division Overview

The Biotechnology Division is the focus of the NIST efforts addressing critical measurement and data needs for the rapidly developing biotechnology industry. The mission of the Biotechnology Division is to provide measurement infrastructure necessary to advance the commercialization of biotechnology. This is achieved by developing a scientific and engineering technical base along with reliable measurement techniques and data to enable U.S. industry to produce biochemical products quickly and economically with appropriate quality control. The Division has established a variety of long-range research programs to maintain critical expertise needed for the development of advanced measurement methods, Standard Reference Materials and databases for use by industry and other research enterprises. It fosters collaboration among NIST scientists conducting biotechnology research, and raises the visibility of the NIST Biotechnology program, which leads to enhanced collaborations with industry, universities and other government agencies.



Biotechnology

G. Gilliland, Chief

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Programs

To plan and guide Division research programs effectively, Division scientists are active in many forums that provide feedback on the long-term and short-term scientific and technological needs for biotechnology commercialization. These activities supplement the high level of Division participation in scientific meetings and topical workshops.

For example, during FY99 the Division continued active participation in the Biotechnology Industry Organization (BIO), in the IUPAC Commission on Biophysical Chemistry, and in the ASTM Committee E-48 on Biotechnology. Division members were also active participants in a number of important workshops held at NIST. The Division also worked closely with the NIST Advanced Technology Program (ATP) in a variety of ways. Division members served as NIST technical representatives in the annual review of a number of active ATP grants. They also presented the results of Division research programs in ATP-sponsored workshops and public meetings. Additionally, Division members have been involved in fundamental research that is directly related to ATP focus areas and also involved in research directly related to specific ATP projects. Throughout the year, the Biotechnology Division continued to play a major role in the planning and co-ordination of Federal biotechnology research through memberships in the working groups associated with the Subcommittee on Biotechnology of the National Science and Technology Council (NSTC). Involvement in these activities assures that the Biotechnology Division is an active participant in the decision making process for prioritizing and directing funding of federal research especially in areas that impact the private sector.

The staff of the Biotechnology Division consists of 69 NIST employees and a comparable number of contract researchers, guest scientists, and postdoctoral fellows. The Division is organized into four groups: (1) **DNA Technologies**; (2) **Bioprocess Engineering**; (3) **Structural Biology**; and (4) **Biomolecular Materials**. In addition, a continued effort was made to develop a program in **Bioinformatics** independent from the other group efforts. A brief overview of the activities of each Group in the Division and highlights of several research programs are given below.

The **DNA Technologies Group** has research efforts to meet goals and objectives in areas of data dissemination, measurement science, and standards. In the area of DNA diagnostics, the group organized a successful TWGNAS (See Technical Working Group for Nucleic Acid Standards) meeting that was held as a follow-on to last year's successful Standards for Nucleic Acid Diagnostic Applications Meeting. As a prelude to that meeting, group staff members received training at a commercial testing laboratory on methodology for diagnosing **Fragile X disease**. Other research in the field of DNA diagnostics is being focused on measurements and standards for molecular cytogenetics and laser scanning cytometry to allow automated slide-based DNA diagnostics. Staff members also received specialized training in the use and production of **"Armored RNA"** for the purpose of providing stabilized DNA or RNA as surrogate standards. Other group activities include continued research in the isolation and characterization of specialized enzymes in support of DNA sequencing, DNA diagnostics, and for industrial enzyme studies.

The Web-based forensic database for **Short Tandem Repeats** (www.cstl.nist.gov/biotech/strbase) is a popular web site. Over 15,000 hits have been recorded since implementation in October 1997. Recent upgrades to the site have added new information on rare variant alleles. This has become an essential touchstone for crime laboratories that need to know if their results are meaningful, or if they are from possibly spurious measurements. The database now includes nearly nine hundred STR references. The group has continued to provide analysis of forensic data for the College of American Pathologists and the National Institute of Justice. Programs in blind proficiency testing for the FBI and a quality assurance program for the Armed Forces DNA Identification Laboratory round out efforts that provide evaluated data and information (see Technical Report 2).

The group's efforts in measurement science have concentrated on the mechanisms of DNA repair enzymes using **GC/Mass Spectrometric** methods and the use of a specially modified capillary electrophoresis instrument to study mechanisms of single-strand conformation polymorphism (CE-SSCP) and to provide optimized methods and standards that permit automated mutation detection. Also as part of the continuing collaboration with and support provided by the National Institute of Justice has led to new activities that will focus on automation of MALDI-TOF Mass Spectrometry for rapid DNA testing and identification of human alleles. Additionally, work in Gene Expression has begun through a CRADA with Gene Logic, Inc.

In the area of standards development, this past year one DNA profiling standard was recertified, and a new SRM for mitochondrial DNA sequencing for use in the nation's crime laboratories was issued (See Technical Report 3). An SRM for PCR-based technologies was also re-issued. These widely used materials are helping to assure accurate measurements in forensic cases. Additionally, work was started for a second mitochondrial standard to allow laboratories to quantify endogenous heteroplasmy. Work on the SRM for p53 tumor suppressor gene mutations was delayed as the plasmids containing various mutations were moved from a patented vector to a publicly available one. Microgram amounts of each plasmid are currently available and interlaboratory testing is being organized. All plasmids have been sequenced to assure fidelity of the materials.

The **Bioprocess Engineering Group** develops of measurement methods, databases, and generic technologies related to the use of biomolecules and biomaterials in manufacturing. Measurement methods and data have been developed in the protein **biospectroscopy** area that will lead to improved understanding of intra- and inter-protein electron transfer processes (see Technical Report 9). This understanding helps industrial biocatalyst development through more efficient utilization of carbon sources (*e.g.*, renewable resources) and nutrients, and in developing new ways to drive organic syntheses such as the stereospecific hydroxylation of pharmaceutical precursors. A group's efforts to develop fluorescence intensity standards have also been continued this year in response to industry and government needs as reflected by the output from recent workshops sponsored and cosponsored by the Biotechnology Division.

In the **biothermodynamics** of enzyme-catalyzed reactions research area, chromatography and microcalorimetry measurements have been combined with chemical equilibrium analysis to develop thermodynamic data for several industrially important bio-transformations (See Technical Report 6). The metabolic pathway by which micro-organisms and plants convert glucose to aromatic amino acids is a current focus of the bio-thermodynamic measurements. This chorismate metabolic pathway is under current investigation by several large chemical companies as an environmentally friendly source of aromatic hydrocarbons. In the **preparative bio-separations** project, electrochromatgraphic/electrophoretic separation equipment and methodology are being applied to the separation of different physical forms of DNA (supercoiled plasmid, relaxed circular plasmid, linear genomic).

Large-scale purification of these materials is an emerging need in industries developing gene therapies and diagnostic materials (See Technical Report 7).

Research projects in **biocatalytic systems** focus on enzyme characterization by site-directed mutagenesis, ^{15}N Nuclear Magnetic Resonance (NMR) spectroscopy, X-Ray diffraction of protein crystals and computational chemistry. These techniques are being used to address focused, industrially important biotransformation problems such as those found in hydroxylation and aromatic amino acid metabolic pathways (See Technical Report 8).

Research by the **Structural Biology Group** at the Center for Advanced Research in Biotechnology (CARB) is focused in four key areas of industrial biotechnology that includes: macromolecular structure determination by X-ray crystallography; molecular structure and dynamics elucidation by modern, high-field nuclear magnetic resonance spectroscopy; physical, molecular and cellular biochemistry; and computational biochemistry and modeling. A balanced program in these four areas has been developed at CARB by recruiting a highly interactive group of scientists through both UMBI and NIST with interests and expertise in the theory and measurement of macromolecular structure-function relationships that underlie virtually all-biological processes. CARB has established profitable interactions with several outside organizations in response to its distinct responsibility as part of a unique collaboration among NIST, the University System of Maryland and Montgomery County, MD. The CARB staff has also engaged in several technical activities that relate directly to NIST's core mission in measurement science and chemical and process information.

The Structural Biology Group research efforts include a broad range of activities. Elucidation of the structure and function of enzymes in the chorismate metabolic pathway continues to be a major area of focus for the X-ray crystallography and modeling groups (See Technical Report 5). This effort, part of the Division-wide activity aimed at the vitally important industrial area of **metabolic engineering**, aims to account for the high specificity and unusual activities of key biosynthetic enzymes in terms of unique structural attributes and theory (See Technical Report 8). Several structures determined for chorismate mutase have spawned a newly proposed mechanism based on theoretical calculations, and biochemical measurements support the model. The NMR group has expanded its focus to evaluate new methods for screening small molecule ligand binding to biochemical targets in an effort to identify novel approaches for **high-throughput screening** (See Technical Report 10). This work is of critical importance for large-scale screening processes that aim to identify high-affinity ligands that disrupt clinically important nucleic acid-protein interactions. Efforts in the biochemical group range from structural, mechanistic, spectroscopic and thermodynamic characterization of important macromolecular interactions. Additionally, new work in the area of **structural genomics** has started to develop large-scale approaches for elucidating the structures and functions of proteins encoded by entire genomes (See Technical Report 11). These activities aim to put descriptive, qualitative biochemical assays on a more quantitative, chemical framework. The computational group continues its focus on developing validated theory for **molecular recognition and interactions**, the basis for the tremendous acceleration of enzymatic catalysis, and factors that contribute to molecular evolution. These activities are also related to the development of tools and databases in bioinformatics that will be beneficial to scientists in both private and public sectors (See Technical Reports 12-17).

The **Biomolecular Materials Group** is examining the underlying need for many applications of biotechnology by the control of biological molecules at interfaces. Chemically controlled surfaces engineered for specific biomolecular interactions are essential components of biosensors, bioelectronics, biocatalytic systems, and many diagnostic devices. **Biomolecular materials** thus influence such diverse applications as pharmaceutical development, health care, environmental pollution monitoring, and chemical manufacturing. A major focus of the group is a rugged artificial membrane system that mimics cell membranes. This **hybrid bilayer membrane** is composed of both artificial and natural lipid components, and is a unique membrane matrix that is both an excellent research tool as well as a commercially important development. Development of the chemistries needed to attach the lipids to a surface has been critical to the progress in this area (See Technical Report 18).

Fundamental understanding of the structural characteristics of biological molecules such as cell membrane receptors, optically active proteins and redox enzymes in this and other biomimetic matrices, and the relationship between structure and functional activity, are key to successful commercial applications. Molecular details are provided by **vibrational spectroscopies** such as infrared and surface plasmon resonance enhanced Raman, and by **nonlinear optical spectroscopies**. The development of models based on electromagnetic wave theory permitted the development of simulated spectra that aid data interpretation (See Technical Report 19). Recent development of **infrared ellipsometry** has been useful for determining lipid orientation and conformation (See Technical Report 20). Similarly, **neutron reflectivity** (in collaboration with the NIST Neutron Research Facility) is providing high-resolution data of these membranes and the location of protein complexes in them (See Technical. In FY99, a new tool, **atomic force microscopy**, was utilized to investigate the molecular details of these synthetic membranes. In addition, a workshop, "Workshop on Scanning Probe Microscopy of Soft Materials" was hosted by the Division in August.

Genetically engineered proteins such as pore-forming **α-hemolysin** are used in systematic studies of how membrane protein structural changes lead to functional changes. The development of stochastic models aid in the interpretation of results and in the design of new experiments. **Sensor applications** development demonstrates the potential commercial usefulness of these proteins and matrices. Synthetic chemistry efforts are providing novel materials to allow assembly of matrices that are even better biomimetics (See Technical Report 22). These tools permit assessment of the activity of biological molecules in high-throughput formats, and to allow the successful engineering, characterization, and application of biomolecular materials for development of future products of biotechnology.

In FY99 **Bioinformatics** program efforts concentrated on the transition of the NSF/DOE/NIH funded **Protein Data Bank** from Brookhaven National Laboratory to The **Research Collaboratory for Structural Bioinformatics** (RCSB) composed of groups from Rutgers University, the University of California San Diego Supercomputer Center, and NIST Biotechnology Division. The move was completed in July. The efforts within the Division have now focused on improving the querying capability of the database resource by improving data uniformity (See Technical Report 23).

The dynamic nature of the biotechnology industry requires an evolving Biotechnology Division that necessitates strengthening existing programs and the development of technical expertise in new areas. The expansion of the DNA technologies effort to include measurement tools and quality assurance standards for **DNA diagnostic measurements** will continue to be a high priority in FY 2000 because of the rapid growth of industrial applications in this area. Included in Division plans are a broadening of the Biomolecular Materials Group's program to include an emphasis on generic needs common to many kinds of surfaces and materials that are components in sensors, processing, diagnostics and other applications. This is a reflection of the importance of biomolecular structures in the future development of biotechnology, particularly in pharmaceutical development and biomanufacturing. Additionally, both the DNA Technologies and Biomolecular Materials Groups will expand their activities into tissue engineering, an important emerging area of the biotechnology industry. In FY 2000 an expanded emphasis will be placed on the development of modeling that relates molecular structure to the properties of biomolecules, biomaterials, and bioprocesses. The use of biological macromolecules in non-biological applications such as **chemical manufacturing** and **environmental bioremediation** continues to be a promising area of biotechnology. The Division will continue to build expertise in bioprocessing technology, with an emphasis on measurements and data to help industry solve generic problems that limit technology development. The Division will maintain its focus on chorismate pathway metabolism in order to develop the information, measurement, and standards requirements for a new area of metabolic engineering. Continued expansion in the area of **Bioinformatics** is also planned for the Division in FY 2000. This will include the development of computational methods for using large biomolecular structure and sequence databases to identify, analyze, or predict the structure and/or function of new biomolecular species. These expanded capabilities will enhance the Division's research programs and aid industry in the efficient use of chemical and biochemical data in the development of new products and processes.

Staff Recognition: James Stivers of the Structural Biology Group was awarded this years CSTL Technical Achievement award for his work on the kinetic mechanism of damage site recognition and uracil flipping by *E. coli* uracil DNA glycosylase.

B. Selected Technical Reports

1. *Microscopic Analysis of Uniformly Arrayed Human DNA Molecules*

P. E. Barker

Objective: Length of the variable cluster of repeats (TTAGGG) at chromosome ends (telomeres) may be a significant biological indicator as an assay for the senescence and growth-status of cells undergoing tissue engineering. Previous studies involved pooled analysis of all repeats or analysis of cells undergoing cell division at metaphase. There has been no method for telomere-repeat length analysis in a cell cycle phase-independent fashion applicable to individual human chromosomes.

Approach: A DNA display technology called DNA combing has been adapted to the analysis of chromosome-length DNA for the investigation of telomere repeat length of individual chromosomes. This involves gentle removal of all cellular constituents except DNA in a method that leaves chromosomal DNA intact. Intact chromosomal DNA is bound to a vertical glass slide while in solution. The preparation is then gently removed from solution by a computer-actuated apparatus at a rate at which the meniscus uniformly stretches the DNA without breaking it. Such DNA preparations are then interrogated by fluorescence *in situ* hybridization with color-differentiated fluorescently labeled probes that simultaneously detect either the repeat motif or the specific chromosome involved.

Results and Future Plans: Single probe and spectral imaging of somatic cell hybrids containing a single human chromosome is being explored as a model system. This work will establish the nature of telomeric repeat variability and the dynamics of repeat loss under different culture conditions in single human chromosomes. Base line data will be expanded to studies of telomeres from identical or non-identical twins to ask whether telomeric repeat dynamics follows a genotype-dependent plan and whether there is chromosome specificity in the loss of repeats from the genome. Such analyses will contribute to applications of telomere dynamics as a cellular barometer for determinations of senescence and viability in cells important to the tissue engineering industry.

2. *NATO Advanced Study Institute Meeting on "Advances in DNA Damage and Repair; Oxygen Radical Effects, Cellular Protection and Biological Consequences"*

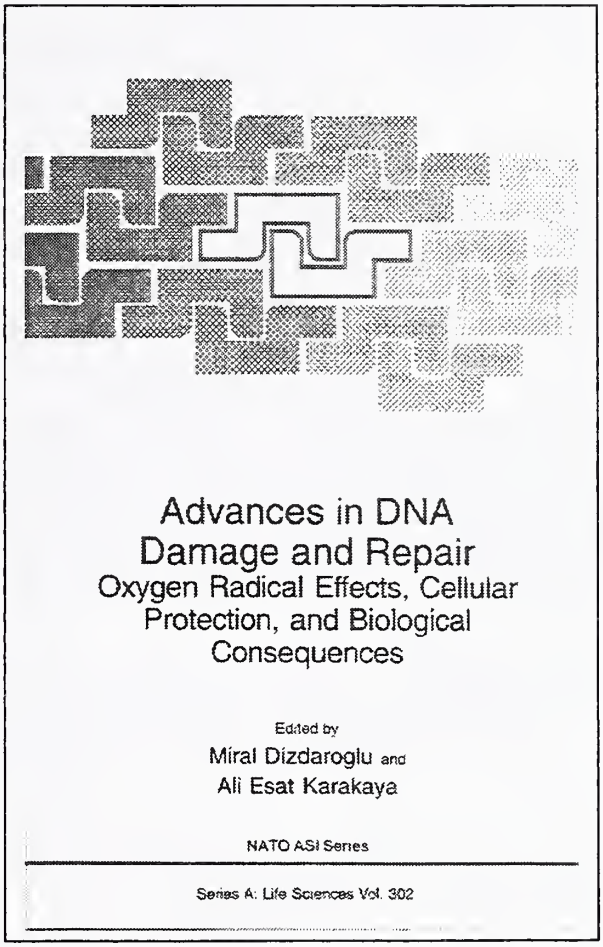
***M. Dizdaroglu** and A. E. Karakaya (Gazi Univ., Ankara, Turkey)*

Objective: To provide the bioanalytical community with a comprehensive collection of recent research on DNA damage and repair.

Problem: DNA damage caused by oxygen-derived species is the most frequent type encountered by aerobic cells. This type of DNA damage is also called oxidative DNA damage and has been implicated in mutagenesis, carcinogenesis, and aging. In cells, there are repair systems that oppose DNA damage. If not repaired, DNA damage may lead to detrimental biological consequences. Therefore, the repair of DNA damage is regarded as one of the essential events in all life forms. In recent years, the field of DNA repair flourished because of new findings on DNA repair mechanisms and the molecular basis of cancer. In 1994, DNA repair enzymes have been named *Science* magazine's "Molecule of the Year."

Approach: The time was ripe to convene a NATO Advanced Study Institute (ASI) of scientists of international standing from the fields of biochemistry, molecular biology, enzymology, biomedical science and radiation biology to present and discuss the basics and new developments of the field of DNA damage and repair. Miral Dizdaroglu and Ali Karakaya organized the NATO ASI on "DNA Damage and Repair Oxygen Radical Effects, Cellular Protection and Biological Consequences." It was held October 14-24, 1997 in Tekirova, Antalya, Turkey.

Results: During the meeting, invited lecturers presented the current understanding and recent developments in this field, and its pertinence to human health. Other participants presented their work in oral and poster sessions. The interactions between the participants contributed greatly to the dissemination of scientific knowledge and the formation of international scientific collaborations. The book of the proceedings contains the papers presented by invited lecturers and the abstracts of the posters presented by the student participants during the meeting. A wide range of topics related to DNA damage and repair is covered by world-renowned experts in thirty-five chapters. Mechanistic aspects of oxidative DNA damage are discussed in several articles that also include discussions of techniques for measurement of DNA damage. Mechanisms of action of a number of DNA repair enzymes are presented in various chapters. These topics include excision repair in eukaryotes, roles of AP endonucleases, genetic stability and aging, actions of DNA glycosylases, bypass of DNA damage by RNA polymerases, nucleotide excision repair in yeast, transcription-coupled DNA repair, DNA repair in premature aging syndromes. DNA damage by nitrogen-derived species and actions of antioxidants against oxidative stress and oxidative DNA damage are also discussed. Several chapters are devoted to the repair of individual lesions of oxidative DNA damage and to enzymes involved in their processing. Biological consequences of these lesions are also discussed in detail. Furthermore, several chapters discuss mechanisms of ionizing radiation-induced damage to DNA and its challenge to repair systems. Other topics include implications of superoxide and nitric oxide interactions in DNA damage and repair, mapping of DNA damage at nucleotide resolution, genotoxicity tests and measurement of DNA repair products in biological fluids. There are more than eighty abstracts in the book presented by student participants in this meeting. These discuss many aspects of DNA damage and repair, mutagenesis, and antioxidants. The proceedings is a comprehensive collection of basic knowledge and recent developments in the field of DNA damage and repair. It will be of great interest to scientists and clinicians who wish to learn more about this rapidly growing area of research. It will also be an essential reference book to students and other young scientists who wish to enter this field of science.



Advances in DNA
Damage and Repair
Oxygen Radical Effects, Cellular
Protection, and Biological
Consequences

Edited by
Miral Dizdaroglu and
Ali Esat Karakaya

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3. *Standardization of Protocols for STR Measurements*

M.C. Kline, J.W. Redman, D.J. Reeder, and D.L. Duewer (839)

Objective: To assess the forensic human identity community's measurement practice in DNA quantity determination and Short Tandem Repeat (STR) typing of mixed-source samples.

Problem: The FBI's Combined DNA Index System (CODIS) enables the sharing of DNA typing information among North American forensic laboratories. Nearly all of these laboratories have recently adopted the CODIS-defined suite of 13 STR loci as their primary DNA typing method. For CODIS to use STR results to identify DNA profiles across jurisdictional boundaries, STR typing must indeed be comparable among all contributing laboratories. Further, for CODIS to be fully utilized, all eligible U.S. forensic laboratories must trust the measurements made by their peers. While the basic DNA extraction, PCR amplification, and STR typing technologies are similar among the forensic laboratories, there is no "standard" STR measurement protocol or reporting policy. Indeed, earlier studies demonstrated many differences among STR measurement protocols and multiple-source sample interpretation policies.

Approach: As part of a continuing series of educational interlaboratory exercises, the NIST Mixed Stain Study #2 (MSS2) was designed to document the "State of the STR Measurement Art" within the North American forensic community. Three separate sets of materials were distributed: **Set 1** three stains on washed cotton sheeting, representing a sexual assault by an unknown assailant; a reference blood stain from a female source, a neat semen reference stain, and a mixed blood/semen stain prepared from the two references plus semen from a second "unknown" male. **Set 2** three stains on washed cotton sheeting, representing a sexual assault; a reference bloodstain from a female source, a neat semen reference stain, and a mixed blood/semen stain where the semen was from a different source than the male reference. **Set 3** five 20 µL DNA extracts in screw-capped vials with DNA concentrations stated to range from ≈0.2 ng/µL to ≈20 ng/µL. Two of these **Set 3** samples were, in fact, replicates. Participants were requested to (1) specify all possible types for all donors represented in each sample of **Sets 1** and **2** for all loci routinely assayed, (2) provide CODIS profile(s) to search for the suspect(s) in the mixed stain samples, (3) estimate the amount of recoverable DNA per sample (ng/stain), and (4) estimate the concentration (ng/µL) of DNA in all **Set 3** samples.

Results: Forty-five local, state, federal, and commercial forensic laboratories reported results in this study (of fifty-two laboratories receiving samples) from January to May, 1999. As anticipated, no participant incorrectly typed any single-donor sample. However, in accordance with laboratory protocols many participants did not attempt to type fully the unknown male in the mixed stain of **Set 1**, and several participants who did attempt to type this unknown male made one or more incorrect assignments. There were large variations in the quantities of DNA recovered and completeness of the differential extractions in the **Set 1** and **2** stains. The median reported DNA concentrations for the TE buffer extracts of **Set 3** agree well with the known gravimetric values but the range in reported values was disturbingly large. Many participants did not clearly identify the replicate quantitative samples.

Future Plans: Because the accurate quantitative determination of DNA in a sample is a critical step in STR typing and for the exchange of PCR-amplification protocols among laboratories, it is proposed to design and conduct an interlaboratory comparison exercise that will focus explicitly on the relationship between DNA quantitation and STR detection amplitude.

4. *A Human Mitochondrial DNA Standard Reference Material for Quality Control in Sequencing*

***B. C. Levin**, L. A. Tully, H. Cheng, and D. J. Reeder*

Objective: The production of a human mitochondrial DNA standard reference material (SRM) for quality control in amplification, sequencing, forensic identification, medical diagnostics and mutation detection.

Problem: DNA from all sources is being sequenced at an unprecedented rate, and the results are being entered into massive genetic databases on the World Wide Web. The problem is that there is little or no quality control to assure that the entered data is correct. Many laboratories are competing to sequence the entire human genome and to determine if various polymorphisms or mutations are associated with specific genetic traits or diseases. The effects of false positives or false negatives can be disastrous. False positives can lead to loss of insurance or worse, *e.g.*, genetic therapy or perhaps other medical interventions to prevent a future illness. In the area of human identification, a false positive could lead to the imprisonment of the wrong individual. False negatives could lead to the freedom of a guilty person or to the manifestation of a disease that might have been prevented. A sequencing DNA SRM would provide the necessary quality control to prevent both false positives and false negatives.

Approach: A human mitochondrial DNA (mtDNA) standard reference material (SRM 2392) has been prepared by NIST to provide quality control to investigators who perform the Polymerase Chain Reaction (PCR) and sequence human DNA for forensic identifications, medical diagnosis, mutation detection or any other. This mtDNA SRM includes extracted DNA from two templates (CHR and 9947A) and all the information necessary to perform PCR amplification, cycle sequencing, gel separation, and data analysis to determine if one is performing these amplification and sequencing procedures correctly. The SRM also includes cloned DNA from the HV1 region of the CHR cell line which contains a long string of cytosines and which is extremely difficult to sequence. The mtDNA sequence of a third human mtDNA template GM03798 is given for comparison, although the DNA is not included as part of this SRM. The sequence information of the fifty-eight sets of unique primers used in the development of this SRM is also supplied.

Results and Future Plans: SRM 2392 is now completed and ready for distribution. Compared to the Anderson sequence, none of the differences found in these three templates correspond to the published mtDNA mutations associated with specific disease. An interlaboratory evaluation of the amplification, sequencing, and analysis of the data from the CHR template conducted by four laboratories indicated that three laboratories experienced no difficulties in obtaining the correct sequence. One laboratory, however, did experience problems, an indication that this laboratory could have used this SRM to improve its techniques. Investigators can now purchase this SRM from NIST and use it as a control when they amplify and sequence their test samples. Corroboration of the SRM results provides quality assurance that any unknown mtDNA is being sequenced correctly and the resulting forensic identifications or medical diagnoses are accurate.

Publication:

Levin, B.C., Cheng, H., and Reeder, D.J. ***"A human mitochondrial DNA standard reference material for quality control in forensic identification, medical diagnosis, and mutation detection,"*** Genomics 55, 135 (1999).

5. *Crystallographic Studies Along the Chorismate Pathway*

J.E. Ladner, P.Reddy, M. Tordova, H. Lee (CARB/NIST), A. Howard (Illinois Inst. of Tech.), E. Eisenstein, and G.L. Gilliland (CARB/NIST)

Objective: To solve the three-dimensional structures of enzymes along the chorismate pathway, in order to contribute to the understanding of how the structure of the enzyme determines its catalytic mechanism, regulation, and biochemical and biophysical properties.

Problem: Unless a detailed three-dimensional structure is available for an enzyme, it is impossible to make precise predictions of the enzymatic mechanism and of how the structure relates to its biochemical and biophysical properties. This in turn will provide insight into the understanding of the overall metabolic pathway.

Approach: A synchrotron X-ray source and cryocrystallographic techniques are being used to obtain the highest resolution data possible in order to obtain a detailed three-dimensional enzyme structure.

Results and Future Plans: Chorismate mutase is an enzyme in the pathway that leads to the synthesis of the aromatic amino acids. The structure of this enzyme from *B. subtilis* was determined a number of years ago for crystals in space group $P2_1$ with 12 monomers in the asymmetric unit. Crystals have been grown that are of space group $P2_12_12_1$ with only a trimer in the asymmetric unit. This structure has been refined at 1.34 Å, and it has been compared to the lower resolution structure found earlier. The trimers in the orthorhombic crystals are cross-linked; the C-terminal tail of each monomer of the trimer interacts with the active site of a neighboring trimer. The active site in the high-resolution structure contains a very strongly held sulfate ion and a glycerol molecule. These ligands come from the crystallization and cryosolvent conditions used, but they make it difficult to include a transition state analog in the crystals. In order to overcome this problem, the mother liquor of the crystals was slowly changed and then the crystal was soaked in a solution that contained the transition state analog. Under these conditions, the crystals changed space group becoming space group $P2_1$ with two trimers in the asymmetric unit. The C-terminal tail of one monomer in each trimer no longer binds to the active site of an adjacent trimer and the transition state analog binds in this freed active site. The resolution of the data for this $P2_1$ crystal form is to 1.74 Å. This structure has been refined and is now being compared to the structure with sulfate and glycerol in the active site.

Recently, a chorismate mutase from *Mycobacterium tuberculosis* has also been isolated, purified and crystallized. High quality crystals of this chorismate mutase have been prepared, and X-ray data to 1.7 Å have been collected. Currently, an attempt is being made to produce a model from related chorismate mutase structures. This model should make it possible to obtain initial phases for the protein structure analysis. The chorismate mutase from *B. subtilis* is a trimer and is a form of an αβ-barrel; it is thought that the chorismate mutase from *M. tuberculosis* is a dimer and is mainly α-helical.

Another enzyme in the chorismate pathway is adc-lyase. Crystals of this enzyme have been grown and X-ray data collected to 1.8 Å. Currently, molecular replacement is being used to solve the structure. After the native structure has been solved, the structure can be used to suggest and test theories concerning the mechanism and biophysical properties of this enzyme.

Publication:
Ladner, J.E., Reddy, P., Davis, A., Tordova, M., Howard, A.J. and Gilliland, G.L. ***"The 1.30-Å Resolution Structure of the Bacillus subtilis Chorismate Mutase Catalytic Homotrimer,"*** submitted.

6. *Biothermodynamics*

***R. N. Goldberg**, Y.B. Tewari, and N. Kishore (Indian Institute of Technology, Bombay, India)*

Objective: The overall aim of this research is the study of the thermodynamics of reactions that can benefit biotechnology.

Problem: Knowledge of the position of equilibrium is essential for predicting the feasibility of chemical reactions and for the optimization of industrial processes. Reaction conditions such as temperature, pH, ionic strength, and co-factor concentrations can substantially affect the position of equilibrium of many biochemical reactions.

Approach: Chromatography, microcalorimetry, thermodynamic modeling, and literature data are used to measure thermodynamic quantities and assemble databases for enzyme-catalyzed reactions. The microcalorimetric capability has been particularly important in allowing for the extrapolation of data to higher temperatures. This effort has also resulted in the development of estimation schemes based upon limited and carefully chosen sets of data.

Results and Future Plans: Recent research has focused on reactions in the chorismate metabolic pathway that has been a focal point of interest because of its potential industrial importance. The thermodynamics of a major portion of this pathway have now been characterized by studying the reactions catalyzed by the tryptophan synthase, prephenate dehydrogenase, prephenate dehydratase, chorismate lyase, chorismate mutase, glutaminase, and tyrosine aminotransferase. This past year, microcalorimetry and high performance liquid chromatography have been used to conduct a thermodynamic investigation of reactions catalyzed by anthranilate synthase, the enzyme located at the branch point in the chorismate pathway that leads to tryptophan synthesis. The overall biochemical reaction catalyzed by native anthranilate synthase is: $\text{chorismate}_{aq} + \text{ammonia}_{aq} = \text{anthranilate}_{aq} + \text{pyruvate}_{aq} + H_2O_l$. This reaction can be divided into two partial reactions involving the intermediate 2-amino-4-deoxyisochorismate (ADIC): (1) $\text{chorismate}_{aq} + \text{ammonia}_{aq} = \text{ADIC}_{aq} + H_2O_l$, and (2) $\text{ADIC}_{aq} = \text{anthranilate}_{aq} + \text{pyruvate}_{aq}$. A mutant form of anthranilate synthase that is deficient in ADIC lyase activity but has ADIC synthase activity was used to study the first of the two partial reactions. The absence of a pure sample of ADIC and the presence of unavoidable side reactions required some innovation in regards to the establishment of the response factor of ADIC, the establishment of the reversibility of the reaction, and the demonstration that equilibrium in fact had been obtained. The results of the equilibrium and calorimetric measurements were analyzed in terms of a chemical equilibrium model that accounts for the multiplicity of ionic states of the reactants and products. These calculations gave thermodynamic quantities at 298 K and an ionic strength of zero for chemical reference reactions involving specific ionic forms. Thus, for the overall reaction (1+2), it was found: $K = 10^{18}$ to 10^{26}, and $\Delta_r H = -(116.3 \pm 5.4)$ kJ mol^{-1}. For reaction (1), it was found: $K = (20.3 \pm 4.5)$, and $\Delta_r H = (7.5 \pm 0.6)$ kJ mol^{-1}. Thermodynamic cycle calculations were then used to calculate thermodynamic quantities for three additional reactions that are pertinent to this branch point of the chorismate pathway. Additional thermodynamic investigations are planned in 2000, and thus, a relatively complete picture of the thermodynamics of this pathway will be obtained.

Publications:

Kishore, N., Tewari, Y. B., and Goldberg, R. N. ***"A thermodynamic study of the hydrolysis of L-Glutamine to (L-Glutamate + Ammonia) and of L-Asparagine to (L-Aspartate + Ammonia),"*** J. Chem. Thermodyn., in press.

Kishore, N., Holden, M. J., Tewari, Y. B., and Goldberg, R. N. ***"A Thermodynamic Investi-gation of Some Reactions Involving Prephenic Acid,"*** *ibid.*, 31, 211 (1999).

Byrnes, W.M., Goldberg, R.N., Holden, M.J., Mayhew, M.P., and Tewari, Y.B. ***"Thermodynamics of Reactions Catalyzed by Anthranilate Synthase,"*** Biophys. Chem., in press.

7. *Preparative Bioseparations*

K.D. Cole and C.M. Tellez (Univ. of Illinois)

Objective: To develop new high-resolution techniques to purify large amounts of different physical forms of DNA.

Problem: Large-scale purification of nucleic acids is necessary to meet the needs of research and development laboratories and for uses as new classes of therapeutic agents. A large number of human gene therapy clinical trials are currently underway. A variety of methods for introducing DNA into cells and tissues are being tested including the use of viruses, cationic liposomes, DNA-protein complexes, and direct injection of DNA. Oligonucleotides designed to inhibit the expression of specific genes show promise as antiviral and anticancer agents. Nucleic acid molecules selected from combinatorial libraries that are capable of high affinity binding to target molecules are examples of ligands that could be used therapeutically or for diagnostic purposes. Production of large amounts of DNA for these experiments is a new challenge in bioprocessing.

Approach: Electrophoresis has proven to be a highly successful method for the separation of nucleic acids on an analytical scale. Electrophoresis in gels and capillaries is a high-resolution analytical method, but scale-up is difficult. Electrochromatography (EC) is proving to be a promising method to scale up the electrophoresis of nucleic acids. EC is the application of an axial electrical field to a chromatography column. This research, which has been carried out in collaboration with Life Technologies Inc., has provided a crude cellular extract that contained supercoiled circular DNA, nicked circular plasmid DNA (relaxed), linear genomic DNA (from host bacteria), RNA, and other cellular constituents. Another approach is gel electrophoresis with reversible media. Gel electrophoresis is another high-resolution technique that is not widely used as a preparative tool because of the difficulty of getting DNA or proteins out of the gel when the separation is achieved. Gel-forming polymers that change to solutions when the chemical environment is changed are being investigated.

Results and Future Plans: Reversible gels that allow the recovery of nucleic acids and proteins after electrophoresis have been developed. These reversible gels are based on the use of the carbohydrate polymer gellan gum. The gels are reversible (return to solution) by changing the ionic environment or pH. During electrophoresis, gellan gum reversible gels have a significant flow of buffer towards the negative electrode (known as electro-osmotic flow). The effect of an additional polymer, gel concentration, and buffer type on electro-osmotic flow has been investigated. By adding a linear polymer to gellan gum gels, a significant reduction in the electro-osmotic flow was observed. The linear polymers used in this study were polyethylene oxide and hydroxyethyl cellulose. Both polymers reduced electro-osmotic flow in the gels, in a manner dependent upon molecular weight. Polymers with high molecular weight were more effective at reducing electro-osmotic flow. Addition of polymers increased the resolution of low molecular weight DNA. Native gellan gum resolved DNA from approximately 50,000 base pairs to 1,000 base pairs. Addition of the polymers resolved DNA to approximately 50 base pairs in some instances. The influence of polymers on circular plasmid DNA was also investigated. Addition of high molecular weight polyethylene oxide reduced the electrophoretic mobility of the nicked circular form compared to supercoiled form. The use of gellan gum gels for protein electrophoresis was done using model proteins.

Publications:

Cole, K.D., Tellez, C.M., and Nguyen, R.B. ***"Addition of Linear Polymers to Reversible Gels: Measurements of Electro-osmotic Flow and Electrophoretic Mobilities of DNA and Proteins,"*** Appl. Biochem. Biotech., in press.

Cole, K.D. ***"Reversible Gels for Electrophoresis and Isolation of DNA,"*** BioTech., 26, 746 (1999).

Tellez, C.M. and Cole, K.D. ***"Method for the Characterization of Size-Exclusion Chromatography Media for Preparative Purification of DNA Restriction Fragments,"*** Biotech. Tech., 13, (1999).

8. *Biocatalytic Systems*

***B. Coxon**, W.M. Byrnes, D.T. Gallagher, M.J. Holden, M.P. Mayhew, A.E. Roitberg, (GeoCenters, Inc.), N. Sari (CARB/UMBI), and V.L. Vilker*

Objective: To develop measurement and modeling techniques for characterizing enzymatic transformations that are important to industrial applications of biocatalysis and metabolic engineering.

Problem: Many new metabolic pathways are being discovered and promoted for use in making chemicals from renewable resources, *e.g.*, glucose from crop spoilage. The separate enzymatic steps in these pathways are not well characterized, when compared to the wealth of knowledge about pathways in mammalian cell physiology. Information such as biocatalytic rates, enzyme structure and function relationships, and genetic and allosteric control of reactant specificity is needed for successful bioprocess development to occur.

Approach: This activity uses molecular biology to engineer and produce proteins, the co-ordinated activity of which is then characterized and modeled by various measurement and computational chemistry techniques. Site-directed mutagenesis, NMR spectroscopy, and X-ray crystallography /diffraction are used to determine and analyze molecular structure. Various molecular modeling techniques are used to interpret function.

Results and Future Plans: Two projects are producing structural information on component proteins to model dynamic interactions during biocatalysis. One project deals with protein electron transfer. This biocatalytic system involves cytochrome P450 (CYP101) and its electron transfer partners, putidaredoxin (Pdx) and Pdx reductase. In this work, the ^{15}N backbone dynamics of Pdx as a function of its redox state was investigated. The results reveal a difference in the dynamic behavior of the reduced and the oxidized forms; the oxidized form has higher mobility in both ps-ns and µs-ms time scales relative to the reduced form. The decrease in the protein dynamics upon reduction is observed almost everywhere in the protein. However, it is more pronounced in regions closer to the Fe-S center, especially in the C-cluster region. This decrease in protein dynamics is in agreement with the results of a redox-dependent dynamics study by amide proton exchange. The significance lies in the fact that reduced Pdx binds to P450cam much more readily than oxidized Pdx. Because binding of Pdx to P450cam requires some loss of degrees of conformational freedom, and because reduced Pdx is more rigid and more readily bound to P450cam in comparison with oxidized Pdx, it is proposed that reduced Pdx populates the same conformational substates in the Pdx-P450cam complex. The decrease in the protein dynamics upon reduction of Pdx, especially in regions closer to the Fe-S center, supports this view. In particular, the drastic change in the dynamics of residue Asp34, which is implicated as a binding site for P450cam, is a very good example of this.

The biosynthetic pathway leading from glucose to chorismate and its aromatic derivatives has major industrial potential and is another system under study. Through collaborations with industry and government laboratories, enzymology and structural investigations of several key components that may be amenable to engineering for improved pathway throughput have been initiated. A database that combines existing structural and other information with new measurements from the Biothermodynamics laboratory has been created. This metabolic pathway is being developed as a model system for bioprocess measurement and modeling technology.

Publications:

Sari, N., Holden, M.J., Mayhew, M.P., Vilker, V.L., and Coxon, B. ***"Comparison of backbone dynamics of oxidized and reduced putidaredoxin by ^{15}N NMR relaxation measurements,"*** Biochemistry-US, 38, 9862 (1999).

Vilker, V.L., Reipa, V., Mayhew, M., and Holden, M.J. ***"Challenges in capturing oxygenase activity In Vitro,"*** J. Am. Oil Chem. Soc., 76, 1283 (1999).

Grayson, D.A., and Vilker, V.L. ***"Kinetic characterization of chiral biocatalysis of cycloarenes by the camphor 5-monooxygenase enzyme system,"*** J. Mol. Cat. B, 6, 533 (1999).

9. *BioSpectroscopy*

A. K. ***Gaigalas*** *and V. Vilker; V. Reipa (Univ. of Cal., Los Angeles); T. Ruzgas and G. Valincius (Vilnius State University, Vilnius, Lithuania); and L. Li (William and Mary College)*

Objective: To apply spectroscopic and electrochemical techniques to characterize biomolecular processes such as electrical or optical energy transfer between redox or fluorophore centers and external surfaces. To measure electron transfer rates and thermodynamic parameters associated with protein redox processes. To develop the scientific basis for producing viable fluorescence intensity standards.

Problem: Commercial application of redox enzyme biotransformations is hampered by the lack of data and models for intra- and inter-protein electron transfer and by the lack of inexpensive and reliable sources of reducing power. The application of fluorescence-based assays in biotechnology for purposes of tracking cellular genetic and metabolic activity has increased dramatically. Further advances in the field are impeded by the absence of fluorescence intensity standards. This knowledge and data is important in the design and development of biocatalysts and biosensors.

Approach: Spectroscopic and electrochemical instrumentation such as linear sweep voltammetry, surface enhanced Raman spectroscopy, electroreflectance spectroscopy, and spectroellipsometry have been developed. These techniques are being used to characterize electrode surfaces, to measure electron transfer in several redox proteins (*e.g.*, putidaredoxin—iron-sulfur redox center, azurin—copper redox center, cytochrome c—heme redox center), and to characterize the stability and quenching of model fluorophore-interface systems.

Results and Future Plans: The accumulation of nitrate in water has become an increasingly severe problem. A recent USGS survey (1995) discovered that the EPA limit for nitrate (10 ppm) is exceeded in 9 % of all domestic water wells, a dramatic increase from 2.4 % found in an earlier survey. At present there are neither methods for *in situ* measurements of nitrite(ate) nor any cost-effective approach to regenerating drinking water contaminated by nitrate. In this report, work is described that is aimed at developing enzyme-coated electrodes, which may lead to nitrite (ate) biosensors, and/or electroenzymatic reactors for detecting and replenishing nitrate-contaminated environmental water bodies. Tetradecylmethylviologen ($C_{14}MV$) was co-adsorbed on gold electrodes with decanethiol in order to mediate electron transfer between the electrode circuit and soluble nitrate reductase enzymes, which catalyze the reduction of nitrate to nitrogen. Surface Enhanced Raman Spectroscopy and *in situ* spectroscopic ellipsometry were used to monitor the structural properties of surface-confined $C_{14}MV$ during its redox conversion. When $C_{14}MV$ is adsorbed by itself, in the absence of $C_{14}MV$ solution species, the *in situ* optical studies showed the loss of initial electroactivity was due to the bipyridine rings being oriented parallel to the electrode plane. This configuration is thought to be unfavorable for the anion (Cl^-) diffusion in and out of the film, which is essential for the redox reaction. The electroactivity in the adsorbed film was restored by co-adsorbing decanethiol ($C_{10}T$) with $C_{14}MV$. This gave an intercalated film with the end-on, bipyridine ring oriented vertically relative to the electrode surface. Intercalated $C_{14}MV$ is stable for several thousand voltammetry scans and was found to be an efficient electron transfer mediator to soluble nitrate reductase despite being embedded in a decanethiolate layer

Publications:

Gaigalas, and A.K. Ruzgas, T. ***"The Role of the Potential Distribution at the Electrode Interface in Determining the Electron Transfer Rate Constant,"*** J. Electroanal. Chem., 465, 96 (1999).

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Li, L., Ruzgas, T., and Gaigalas, A.K. ***"Fluorescence from Alexa 488 Fluorophore Immobilized on a Modified Gold Electrode,"*** *ibid.*, in press.

10. *Screening Ligand Binding to RNA using Fluorescence and NMR Spectroscopy*

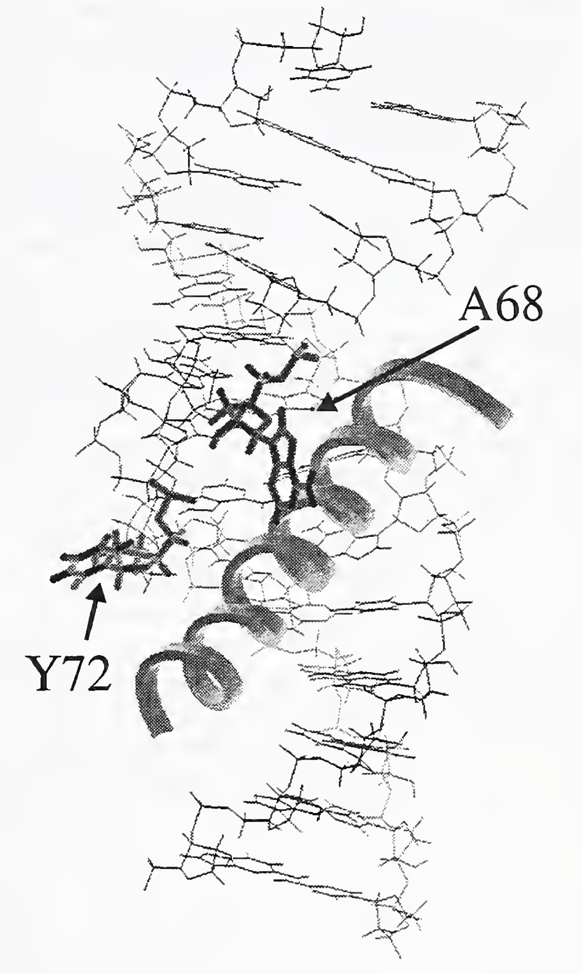
J.P. Marino, J.T. Stivers and K.A. Hickey (CARB/NIST)

Objective: To develop general approaches for detecting and quantifying nucleic acid-protein interactions that can be used in high-throughput screens and for obtaining rapid structural information on these complexes to guide rational drug design.

Problem: The development of specific inhibitors of protein-nucleic acid complexes is of significant interest since these complexes provide new and potentially powerful targets to regulate or inhibit gene expression and viral/bacterial infection. For instance, the inhibition of proteins that are involved in mRNA processing, DNA replication and transcription, or DNA damage repair could provide powerful new drug targets to combat viral infections, fight cancer, regulate gene expression, or enhance the effectiveness of existing chemotherapeutic agents. A combined fluorescence/NMR technology based approach to provide a generally useful method for screening and optimizing inhibitors of nucleic acid-protein complexes has been developed.

Approach: The approach utilizes structural information obtained from NMR to guide the placement of the fluorescent nucleotide analog, 2-aminopurine 2'-O-methyl riboside (2-AP), which is extremely sensitive to changes in nucleic acid structure, into defined positions in the RNA target sequence. The utility of the method has been demonstrated using the well-characterized RNA-peptide model system derived from the HIV-1 Rev protein interaction with the Rev Responsive Element (RRE). Rev is an important HIV-1 regulatory protein that binds RRE within the *env* gene in HIV-1 RNA genome and thus is a potential therapeutic target in the treatment of HIV-1 infection. Using this approach, 2-AP has been incorporated into the RRE RNA sequence in two non-perturbing positions (A68 and U72, see the figure) such that the binding of both Rev peptide and small aminoglycoside ligands could be followed by fluorescence methods. This method enables the identification and quantification of aminoglycoside-binding events to RRE that are both competitive and non-competitive with Rev peptide binding. In addition, NMR measurements of chemical-shift perturbations have provided complementary structural information that has allowed the localization and characterization of aminoglycoside interactions with RRE and the RRE-Rev complex.

Results and Future Plans: Rev peptide binding to the RRE-72AP variant results in a 2-fold fluorescence increase that provides a useful signal to monitor this binding interaction ($K_D = 20 \pm 7$ nM). Using the RRE-68AP and 72AP constructs multiple classes of binding sites for the aminoglycoside neomycin have been detected. One tight site ($K_D = 210 \pm 40$ nM) is not inhibitory to Rev binding, a weaker site ($K_D = 2.9 \pm 0.9$ μM) inhibited Rev binding in a competitive fashion, and a much weaker class of sites ($K_D = 60 \pm 9$ μM) is attributed to nonspecific binding. NMR measurements have provided structural information that suggests that the tight and inhibitory neomycin binding sites on RRE are located on the stem and bulge region, re-



NMR derived RRE Rev complex structure with positions U72 and A68 within the Rev high affinity binding site highlighted.

spectively. The results suggest a new strategy for future drug design involving the tethering of new functional moieties on neomycin thereby converting the tight binding site into an inhibitory site for Rev binding.

In general, it has been shown that the fluorescence methodology is faster, more sensitive and more reliable when compared to other methods, such as filter binding, gel shift, and chromatography, that are commonly used to detect and quantify nucleic acid-protein complexes. The NMR-guided approach for the rapid, optimal placement of fluorophores in these types of nucleic acid-protein system has also been validated. This method should therefore provide a generally useful paradigm for developing high throughput screens for the detection of nucleic acid-protein complexes.

11. *High-Throughput Protein Expression and Purification for Structural Genomics*

N. ***Bonander*** *(CARB/NIST), J. Toedt, and E. Eisenstein (CARB/UMBI)*

Objective: To design a convenient and reliable strategy for the expression and purification of proteins encoded for by open reading frames of unknown function.

Problem: Recent developments in automated techniques for DNA sequencing have led to an explosion of information on the complete sequences for the genomes of several organisms. Strikingly, as each new organism's genome is analyzed it has been found that almost one third of the putative open reading frames, although conserved among several organisms, encode for hypothetical proteins of no known function. The goal of this project is to elucidate the function of these proteins by determining high-resolution, atomic structures. A significant obstacle to this approach is that it is absolutely dependent upon the availability of large quantities of highly purified recombinant material for many different proteins.

Approach: The approach has focused on using a small subset of protein expression vectors that typically yield high levels of recombinant protein expression in bacterial culture. Vectors that express recombinant proteins containing a removable affinity tag are obvious first candidates for screening and rapid purification studies. It is also of interest to examine levels of native expression, especially for proteins that are destabilized when expressed with a purification tag.

Results and Future Plans: About two-thirds of the initial trial set of 50 hypothetical proteins have been successfully expressed at high levels and purified to homogeneity. Yields range from a few to several hundred milligrams of material per liter of bacterial culture, certainly ample amounts for structural analyses. The most significant challenges concern maximizing expression; conditions for high-level expression vary widely for the various targets. Surprisingly, affinity purification of many candidates was not straightforward, and alternative approaches were needed for many proteins. Future work will focus on adapting methods for classical protein purification to the simultaneous purification of a large number of recombinant proteins.

Structure of a protein of unknown function (HI1434) encoded from an open reading frame of Haemophilus influenzae.

12. *Mapping Protein Binding Sites on Rhodopsin through Expression of Soluble Interacting Domains*

K.D. Ridge (CARB/NIST), T. Ngo, and
N.G. Abdulaev (CARB/UMBI)

Objective: To identify regions in rhodopsin that interact with signaling proteins upon light-activation.

Problem: The dim-light photoreceptor rhodopsin is a prototypical member of the superfamily of G-protein coupled receptors sharing the seven-transmembrane-helix structural motif that regulate a variety of sensory, hormonal, and neural responses. Rhodopsin is composed of the apoprotein opsin, a single polypeptide chain of 348 amino acids, and a covalently linked 11-cis-retinal chromophore. While the chemical aspects of rhodopsin structure have yielded to investigation, knowledge about the conformational changes that occur upon light-activation of the photoreceptor are far from being understood at the molecular level. The goal of this research is to understand signal transduction in the visual cell by localizing solvent exposed regions on light-activated rhodopsin that trigger the binding and activation of soluble signaling proteins.

Approach: The putative interacting surface(s) for various signaling proteins were reconstructed by inserting fragments corresponding to solvent exposed cytoplasmic regions of rhodopsin either singly, or in combination, onto a surface loop in the soluble *E. coli* enzyme thioredoxin. The expressed and purified fusion proteins were tested for their ability to mimic the cytoplasmic surface of rhodopsin in activating three signaling proteins (G-protein, rhodopsin kinase, and arrestin) that mediate visual transduction.

Results and Future Plans: Biochemical studies show that some of the fusion proteins effectively mimic rhodopsin in activating G-protein or competing with the light-activated rhodopsin/G-protein interaction, in supporting rhodopsin kinase mediated phosphorylation of a carboxyl-terminal rhodopsin peptide, or phosphorylated peptide stimulated arrestin binding. These results suggest that rhodopsin's cytoplasmic loops on the thioredoxin fusion proteins are capable of folding to a functional conformation similar to that of light-activated rhodopsin. Further, these studies should allow a more detailed analysis of the rhodopsin:signaling protein interaction(s) using state-of-the-art biophysical and structural measurement methods.

13. *Thermodynamic Stabilities of PNA/DNA and DNA/DNA Duplexes*

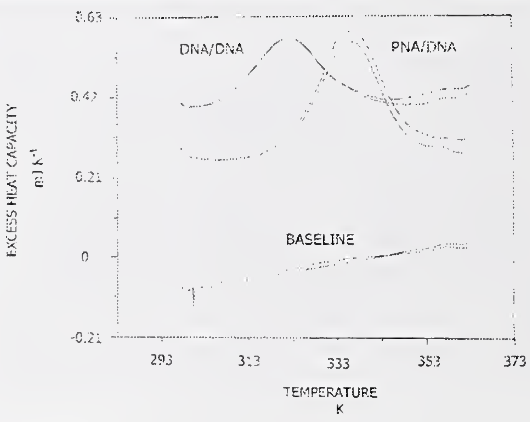
F. P. Schwarz and M. C. Chakrabarti (CARB/NIST)

Objective: To develop and ascertain methods for determining the thermodynamic stability of PNA/DNA and, for comparison, the corresponding DNA/DNA duplexes employing three different experimental methods. To build-up a thermodynamic database on the thermal stabilities of the duplexes, which can ultimately be employed to develop a model for prediction of these stabilities from sequence information.

Problem: PNAs are synthetic analogues of DNAs where the phosphoribose backbone of the DNA is replaced by an ethylglycine-peptide-linked backbone. PNAs are neutral, inert, nontoxic, and bind to their complementary DNA sequences to form highly stable PNA/DNA duplexes, more stable than their corresponding DNA/DNA duplexes. They can, thus, be used in a wide range of applications including enhancing PCR amplification of targeted DNA sequences, increasing the detection sensitivity of targeted DNA strands in MALDI-TOF analysis, inhibiting the transcription of specific genes in gene therapy, and increasing the detection level in DNA chip diagnostics. Thermodynamic models predicting the stability of the PNA/DNA duplexes from sequence information are necessary to design PNA sequences for use in these applications.

Approach: Isothermal titration calorimetry (ITC) measurements on the formation of the duplexes at ambient temperatures and differential scanning calorimetry (DSC) measurements and UV melting measurements on the dissociation of the duplexes at high temperatures are being employed to determine the free energy, enthalpy, and entropy of formation of the duplexes. In ITC, the heat released upon duplex formation at any temperature is measured, while in DSC, the heat absorbed at the duplex melting temperature is measured. UV melting involves measuring the change in the hyperchromicity at 260 nm of the duplex as it dissociates upon increasing the temperature of the duplex. The determination of the thermodynamic quantities of duplex formation from the calorimetry measurements does not involve any assumptions regarding the nature of duplex formation, whereas the widely used UV melting method assumes that the dissociation of the duplex is a simple two-state process. UV melting and DSC measurements are also employed to determine any changes in the conformations of the single strands between ambient and the melting temperature, which would contribute to the thermodynamic quantities of duplex formation.

Results and Future Plans: Determinations of the thermodynamic quantities for the formation of the 10 base pair duplexes and ITC measurements on the 8 base pair duplexes have been added to the data base. Thermodynamic quantities on formation of the 10 base pair duplexes when extrapolated from the DSC and UV melting measurements do not agree with the ITC results at ambient temperatures. UV melting measurements on the PNA and DNA single strands show that the conformations of both strands at ambient temperatures and at the melting temperature are not the same thermodynamically. These conformational differences make contributions to the thermodynamics of duplex dissociation, which can account for the discrepancies between the extrapolated thermodynamic values from the UV melting and DSC measurements and the direct ITC measurements on duplex formation at room temperature. This is important since for the past thirty years, the UV melting measurements have been used to determine DNA to DNA binding affinities at ambient temperatures based on the *incorrect* assumption that the single strand states are thermodynamically the same at the two temperatures. Presently, with DSC and ITC, the thermodynamics of the thermally induced conformational changes in the single DNA and PNA strands are being determined and added to the database. Interestingly, these contributions appear to be more important for the longer 10 base pair sequences than for the shorter 8 base pair sequences. Research is now being focused on whether the single strand thermodynamic contributions are sequence dependent and on more accurately extrapolating the UV melting results to ambient temperatures. Upon completion of the DSC and UV measurements on the 8 base pair sequences, this three-method approach will be applied to the longer 12 base pair duplexes to determine the effect of sequence length on duplex formation. In a CRADA with P. E. Biosystems, synthesis of the PNA strands has been as well as measurements on PNA/RNA duplex formation.



Repeated differential scanning calorimetry scans of the dissociation of a DNA/DNA 10-mer duplex and its corresponding PNA/DNA 10-mer duplex in solution.

Publications:

Schwarz, F.P., Robinson, S. and Butler, J. M. ***"Thermodynamic Comparison of PNA/DNA and DNA/DNA Hybridization Reactions at Ambient Temperatures,"*** Nucleic Acids Res., in press.

Chakrabarti, M.C. and Schwarz, F. P. ***"Thermal Stability of PNA/DNA Duplexes by Differential Scanning Calorimetry,"*** *ibid.*, in press.

14. *Resonance Raman Spectroscopic Measurements of Large Enzyme-DNA Complexes: Quantifying Electronic Rearrangements Important for Enzymatic Catalysis*

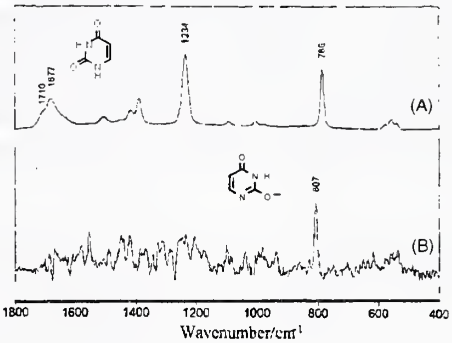
J.T. Stivers (CARB/NIST)

Objective: To develop cutting-edge spectroscopic measurement methods that provide the basis for understanding the forces and interactions that lead to the large catalytic rate enhancements (10^{12} to 10^{18}-fold) and specificities ($>10^{6}$-fold) of enzymes that act on DNA substrates. To provide measurements and models that lead to improvements in drug design, and the bioengineering of improved catalysts for biotechnology and medical diagnostic applications.

Problem: Enzymes that act on DNA substrates have become powerful and indispensable tools in the current biotechnology revolution. These enzymes are used in medical diagnostic applications, the synthesis of novel reagents and drugs, and many basic research applications that ultimately lead to new biotechnology products. Thus, it is not an exaggeration to state that this revolution is *driven* by these enzymatic activities. Although nature has provided many useful enzymatic activities without intervention by biochemists, it is highly desirable to develop methods and models that allow for rational design of new enzymatic activities. Such a goal is not trivial and requires high-resolution structural methods, such as hetero-nuclear NMR and X-ray crystallography, as well as spectroscopic measurements that allow detailed characterization of the electronic rearrangements in substrates and products when bound in the active site environment of enzymes.

Approach: Spectroscopic investigations are being expanded into the prototypic DNA repair enzyme, uracil DNA glycosylase (UDG), using Raman spectroscopy, a laser-light scattering method. Recent improvements in spectrometer performance now allow the study of complex enzyme systems using small sample sizes (50 µL) and low concentrations (100 µM). This method, which intimately probes the vibronic states of molecules, is complementing ongoing spectroscopic studies that already include X-ray diffraction, heteronuclear NMR and fluorescence methods. This multidisciplinary approach is allowing the unraveling of the detailed basis for the catalytic power of this enzyme at the atomic level. The development of new spectroscopic measurements that probe the electronic features of the UDG active site will provide key data that allow the rational design of inhibitors of this enzyme.

Results and Future Plans: Using Raman spectroscopy it has been recently established that UDG changes the electronic properties of the bound product uracil at pH 7.5 so as to mimic that of the free uracil monoanion. This requires that the enzyme active site environment has stabilized the uracil anion by about 20 kJ/mol relative to an aqueous environment. This result provides a key insight into the interactions that lead to tight and specific binding of uracil and suggests electronic features that should be incorporated in potential inhibitors of UDG. The rational design of antiviral agents targeted to UDG, and the bioengineering of its active site to recognize and remove other types of unnatural DNA bases are future goals of this work.



Resonance Raman difference spectra for (A) free uracil and (B) uracil bound to UDG in the presence of abasic DNA at pH 7.5. The absence of the carbonyl bands at 1700 cm^{-1} and the appearance of a new band at 807 cm^{-1} in the UDG complex indicates that the uracil is anionic in the UDG complex.

15. *Transition State for Hydrolysis of Trimethylphosphate in Phosphotriesterase*

S. Worthington and M. Krauss (CARB/NIST)

Objective: To modify theoretically the active site of an enzyme and to predict structures and analyze the binding and reactive behavior for native and alternate substrates.

Problem: Determination of X-ray structures of enzyme-substrate complexes that are relevant to the mechanism of reaction is dependent on the production of an appropriate crystal that is often difficult or impossible to obtain. Engineering the chemistry of the enzyme by mutating active site residues requires many structural determinations and thus, the production of numerous appropriate crystals. Theoretical methods are now being developed to determine active site structures for the modified enzyme starting from any native structure with a bound substrate relevant to the native chemical mechanism. The fundamental assumption is that the active site bound to the native substrate is relatively rigid. The active site binding of reactant, transition state, and product analogues can now be obtained where these molecules are optimized quantum mechanically. Also, selected mutant residues are optimized in conjunction with the reacting molecules. These procedures substantially leverage a single x-ray structure into a wide range of relevant structures. This method also allows the conservative modification of this active site into another with similar but not identical chemical behavior or with unusual substrates.

Approach: A powerful new method for calculating large molecular systems has recently been developed and is now being applied to biomolecules. The enzyme, for example, is divided into three regions, the chemically active substrate and residues, its immediate environment, and the rest of the protein. The first two regions constitute the active site of the enzyme. The chemical reaction must be treated quantum mechanically but even this smaller region can be too large for such a calculation to be tractable today. The chemically active region is treated by *ab initio* quantum chemistry while the immediate environment or spectator region is treated using effective fragment potentials (EFP) that represent the electrostatic, polarization, charge transfer, and repulsive interactions to the *ab initio* model. The final outer region would be represented by charges for the electrostatic, polarization, charge transfer, and repulsive interactions to the *ab initio* model. The EFP integrals and gradients have been implemented in the GAMESS quantum chemistry code allowing optimization of the structures of the chemically active region in the immediate protein environment. The chemically active region can include protein residues as well as substrate molecules allowing the analysis of mutants as well as native structures. A wide variety of EFPs have been generated to represent protein residues including both the backbone and side-chain.

16. *Modeling Molecular Recognition*

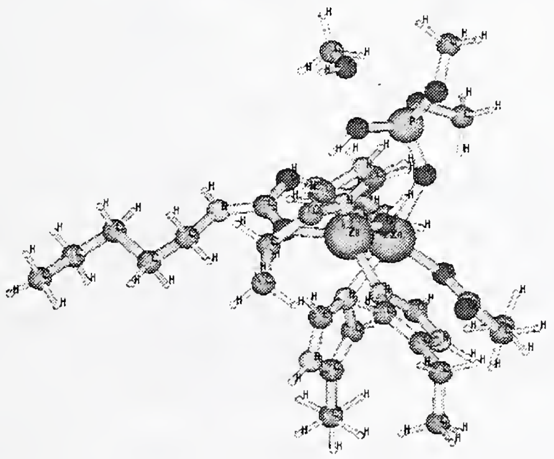
M.K. Gilson, K. Mardis, and J. Potter (CARB/NIST)

Objective: To develop efficient algorithms and software for computing the affinities of noncovalent complexes in solution.

Problem: The noncovalent association of molecules in solution is of fundamental importance in biology and chemistry. Software for predicting what molecules will bind each other, and how tightly they will bind, is needed by industry in order to avoid time-consuming and expensive binding measurements. Such software will be useful for designing molecules targeted to bind specific other molecules. Examples are drug molecules that work by binding enzyme active sites, and smaller host molecules that can be used for chemical separations. However, predicting binding affinities with sufficient accuracy and speed is difficult because of the complexity of the systems.

Approach: The approach has two main elements. The first is the use of simplified—and therefore computationally fast—treatments of the solvent that nonetheless capture much of the relevant physical chemistry. The generalized Born/Surface Area (GB/SA) solvation model is bound to be fast and can readily be corrected toward the results of more rigorous models. The second element of the approach is the use of a novel "mining minima" algorithm. This algorithm rapidly identifies the most stable conformations of a small-molecule system and then computes the contributions of these conformations to the overall free energy. It combines an efficient energy-optimization method with careful treatment of the underlying statistical thermodynamics.

Results and Future Plans: Strong agreement with experiment for a range of small-molecule systems has been found. The most recent case studies involve the association of adenine with a series of seven synthetic adenine receptors and the association of cyclic urea inhibitors with HIV-1 protease. The present methodology also has proven useful for elucidating the mechanisms by which oxygenated aromatic compounds interact with an ester polymer sorbent used in chemical separations. This project has been a collaboration with Dr. Gregory Payne's experimental group at the Center for Agricultural Biotechnology. Future plans include further enhancements of the implicit solvation model and generalization of the mining minima algorithm to make it applicable to molecules with flexible rings and restrained loops.



Active site of phosphotriesterase, all-electron complex with transition state for hydrolysis of trimethylphosphate.

17. *Re-evaluating the Influence of Mutation Biases on Evolutionary Direction*

A. Stoltzfus (CARB/NIST) and L.Y. Yampolsky (CARB/UMBI)

Objective: To develop and explore a token population genetics model that clarifies the influence of biased mutation on evolutionary change.

Problem: In the 1930's, mathematical population geneticists argued that biases in mutation could not influence the course of evolutionary change, given that selection coefficients are generally much larger than mutation rates. This argument is an important one for the development of evolutionary theory and is due for re-examination because it assumes linearity of gene effects and equilibration over time. The assumptions are unrealistic, and furthermore, modern DNA sequence divergence data indicate that mutation biases strongly influence the course of evolution, a result that, under present theory, is consistent only with neutral evolution. Hence, the overall goal is to explore the non-linear and non-equilibrium conditions under which biases in variation influence non-neutral evolution.

Approach: The approach is to study the simplest model in which both natural selection and biases in variation might bias outcomes. First consider a case in which mutation and selection at the A or B locus in an ab population can move the system to one of two fitness peaks, Ab or aB, one of which is more favorable with respect to selection (*i.e.*, the higher peak), and the other more favorable with respect to mutation (see table below; **s1 > s2**, and **u1 < u2**; the non-linearity in this scheme is that AB combines both changes, yet is not advantageous). The classic approach would suggest that selection will always move the system to the higher peak.

Results and Future Plans: A stochastic model using computer simulations and a deterministic model using difference equations have been explored. The most important result is that, in the stochastic model, when variants are rare the bias in which peak the system reaches, Ab/aB, is simply **(s1/s2) * (u1/u2)**. For instance, with a 4-fold bias in mutation favoring one peak, and a 2-fold bias in selection favoring the other peak, there is a 2-fold bias in outcomes toward the mutationally favored peak. This is a very important result. The deterministic approach, somewhat surprisingly, does not always confirm the classic view either. The stochastic model will likely converge on the deterministic outcome as population size is increased and variants are common (*i.e.*, **uN > 1**). In the future a more realistic case will be explored: models of protein sequence evolution with non-linearity in form of amino acid interactions, and mutation biases in GC/AT ratio.

genotype	Fitness	mutation
$\underline{Ab}$	1	-
Ab	1 + s1	u1
$\underline{AB}$	1 + s2	u2
$\underline{AB}$	1	u1*u2

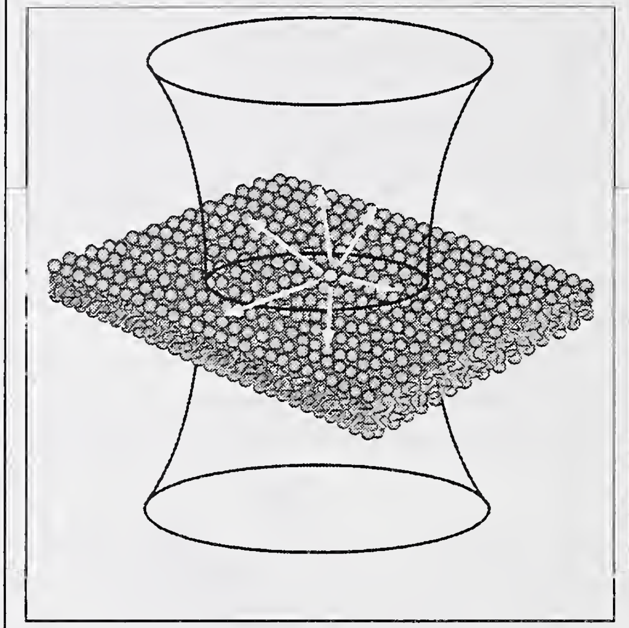
18. *Optically Interrogating Single Molecules in Bilayer Membranes*

***D. Burden** and J. Kasianowicz*

Objective: A new measurement technology that combines single-molecule fluorescence spectroscopy with single ion-channel electrophysiology is under development. The technique will enable the structure and function of individual membrane-incorporated molecules to be simultaneously interrogated with light and electricity in real time. As a first step toward this long-range goal, a highly sensitive scanning confocal fluorescence microscope was coupled to a planar lipid bilayer apparatus, allowing basic optical studies on the behavior of fluorescently labeled lipids at the single molecule level to be conducted.

Problem: Quantification of single-molecule Brownian motion in lipid bilayers has the potential to yield the most accurate diffusion measurements possible. Traditional techniques, such as fluorescence photobleaching and recovery, nuclear magnetic resonance, and electron spin resonance require a relatively high label concentration (typically 0.1-1%) to generate a sufficient signal-to-noise ratio. The disturbances caused by the presence of the label can contribute to varying results for diffusion measurements performed with different techniques on the same membrane system. Experiments employing single-molecule instrumentation enable measurements to be conducted at a much reduced label concentration (below 0.00005 %); thus, label-induced perturbations are virtually eliminated.

Approaches: A combination of confocal microscopy and electrophysiological techniques are being utilized to monitor the behavior of lipid membranes containing single or multiple fluorescently labeled lipids and proteins.



Optical field impinging a lipid bilayer.

Results and Future Plans: Diffusion measurements reveal a surprisingly large optical trapping potential on single molecules. At low photon flux (~10 kW/cm^2), an optical trapping effect appears that is ~5 × 10^6 larger than theory predicts for single lipid molecules in solution and 100 times larger than predicted for lipids in a bilayer. Interestingly, at moderate excitation powers (~300 kW/cm^2), the laser is capable of guiding the diffusive motion of individual fluorophores as the beam scans across the membrane plane.

These findings have significant implications for a variety of techniques that apply large optical gradients to membranes. Confocal microscopy has been widely used to study ensembles of fluorescent molecules in cells and membranes in the past. The technique has only recently been applied to the study of membrane-kinetics at the single-molecule level. In order to make accurate diffusion measurements in cells or model membranes, precautions must be taken to avoid the apparent perturbations (*e.g.*, photobleaching and optical trapping) caused by high optical fields. Additionally, the ability to guide molecules individually or as small groups has exciting implications for nanofabrication, membrane patterning, and controlled-release drug delivery. In the future, it might be possible to enhance the trapping effect by using molecules with multiple fluorescent labels, or labels with improved photostability. This type of optical "handle" would be orders of magnitude smaller than that currently used for optical trapping and manipulation and would allow the molecule under control to better approximate its native behavior.

Publication:
Burden, D. and Kasianowicz, J., "***Optically Guiding Single Small-molecule Diffusion in Planar Lipid Bilayers,***" submitted.

19. *A Combined Molecular Dynamics Simulation and Infrared Spectroscopic Study of Alkane Chains in an Aqueous Environment*

***J.B. Hubbard**, C. Meuse, V. Simmons (CSTL Graduate Fellow), J.C. Rasaiah (Univ. of Maine), and R.D. Mountain (838)*

Objective: To construct a statistically reliable equilibrium classical molecular dynamics simulation of surface-tethered alkane chains in contact with a realistic aqueous molecular environment. To extract thermophysical, spectroscopic, and dynamic information that then can be related to experimental and numerical modeling studies.

Problem: A system consisting of several hundred surface-tethered complex hydrocarbon molecules in contact with several hundred water molecules requires temporal resolution at the femtosecond time scale and a careful consideration of how to deal with the long-range forces due to the presence of the electrical charge that resides in current models for molecular water. Nanosecond timescales are typically required for equilibration while elaborate time-consuming numerical treatment of superimposed Coulomb potential effects can severely tax the capabilities of modern computational facilities.

Approach: An idealized model describing the surface-alkane chain interaction along with a united atom was adopted, rather than a fully atomistic, representation of the intra- and inter-molecular forces. In addition, selected spectroscopic features, such as C-H vibrations in the terminal methyl groups, have been incorporated for the purpose of establishing direct contact with infrared experiments and phenomenological numerical modeling. In order to reduce simulation time and processor requirements, a thoroughly tested point charge model for water has been introduced with the additional simplifying feature of a finite-range cut-off in the Coulomb potential. This simulation of a composite surface-alkane chain/aqueous system is currently in the process of being fine-tuned. In order to accelerate the approach to equilibrium, a modular alkane chain/aqueous phase annealing procedure has been devised in place of more conventional global equilibration techniques.

Results and Future Plans: A series of systems comprised of 225 sulfur-terminated, surface-tethered, 6, 7, 8, and 18 carbon-atom alkane chains has been simulated over a range of temperatures and surface packing densities. Relative to the flat surface, atomic number density profiles, orientation distribution profiles, potential energy density profiles, and atomic level stress profiles have been obtained with a high degree of statistical reliability. Moreover, C-H relative velocity correlation functions for the terminal methyl groups have been calculated, normal and surface-parallel projections have been extracted, power spectral densities of these infrared active vibrations have been computed and carefully compared with high resolution polarized infrared spectroscopic data, and certain key spectral features have been fine-tuned so as to approximately match experimentally determined selected infrared features.

Plans include the possibilities of carboxylating the terminal methyls, the introduction of hydrophilic spacers such as ethylene oxides into the alkane chain terminus and interior so as to simulate surfaces which are highly resistant to the non-specific adsorption of proteins, the introduction of monovalent and even divalent cations and anions into the aqueous/hydrophobic environment, the introduction of electrical fields, and the determination of electrochemical potential profiles together with the simulation of the surface dynamic electrical impedance.

20. *Infrared Spectroscopic Ellipsometry and Alkylated 1-Thia(ethylene oxide) Monolayers*

C. Meuse, D. Vanderah, and V. Silin (Georgetown Univ.)

Objective: To develop measurement techniques for the quantitative characterization of novel materials to determine the structure-activity relationships of model biological surfaces.

Problem: Interest in trans- and integral membrane proteins for sensor and biomedical applications continues to increase. Future technologies based on these compounds will require supporting matrices approximating or "mimicking" natural bilayers. Various strategies such as hybrid bilayer membranes (HBMs) consisting of a bound inner layer of n-alkanethiol self-assembled monolayers (SAMs) on Au and an outer layer of phospholipid have been utilized to constitute effective supporting matrices at or near a surface. However, protein incorporation into HBMs with n-alkanethiol SAM inner layers is not be optimal because alkanethiol layers are highly ordered and do not provide the proper polar environment for water and extra-membranous protein segments.

Approach: Synthetic, measurement, and analytical expertise were combined to construct and characterize SAMs with unique structures for utilization in model biological surfaces. Measurement techniques such as infrared spectroscopic ellipsometry (IRSE) for the quantitative characterization of model biological surfaces such as hybrid bilayers are being developed. IRSE measures the relative intensity of and the phase difference between the parallel and perpendicular components of a polarized electric field vector interacting with a sample, as the complex optical density function. The advantage of the complex optical density function is that it relates the properties of the polarized electric field vector to the supporting surface instead of the plane of incidence. This simplifies the calibration procedure and clarifies what is being measured since the specific properties of the supporting surface and experimental setup are removed from the results. By matching measurements and electromagnetic wave theory predictions, a description of the thickness and molecular structure can be obtained. Wider application of these techniques would allow the quantitative analysis of the orientation and molecular composition of anisotropic samples and assist newly developing applications in pharmaceutical screening, biosensors, tissue analysis, and disease diagnostics.

Results and Future Plans: IRSE was utilized to characterize two sets of monolayers. These measurements were compared to electromagnetic wave theory simulations to reveal thicknesses of 0.8 nm, 1.0 nm, and 2.0 nm ± 0.2 nm for hexanethiol, dodecanethiol, and hexadecanethiol monolayers, respectively. These values are similar to those obtained using visible ellipsometry. However, they did not require the inclusion of an extra, unknown layer to describe the interface between the gold and the alkanethiol.

The structures of the SAMs of alkylated 1-thiaoligo(ethylene oxide) [$HS(EO)_xR$, where $R = C_{10}H_{21}$, $EO = -CH_2CH_2O-$, and $x = 4-8$] were synthesized and characterized on gold using visible spectroscopic ellipsometry and infrared spectroscopic ellipsometry. The SAMs of the alkylated 1-thiaoligo(ethylene oxides) were prepared on polycrystalline gold. The EO segment was found to adopt a 7/2 helical structure oriented normal to the substrate for $x = 5-7$. Different structures were found for the other compounds in this series. When $x = 4$, the EO segment adopts a predominantly trans-extended conformation. Whereas, when $x = 8$ the EO appears to be disordered with some helical conformation. Monolayer thicknesses determined by SE are consistent with these structural changes. A significant increase in disorder with extension of the hydrophilic segment by two ethylene oxide units from 6 to 8 is potentially valuable for protein reconstitution in supported hybrid bilayer constructs to provide a disordered polar region near the Au for HBM constructs.

Publications:

Meuse, C.W. ***"Infrared Spectroscopic Ellipsometry of Self-Assembled Monolayers,"*** submitted.

Vanderah, D.J., Pham, C.P. Springer, S.K., Silin, V., and Meuse, C.W., ***"Characterization of a Series of Self-Assembled Monolayers of Alkylated 1-((Thia)ethylene oxides)$_{4-8}$ on Gold,"*** submitted.

21. *Planar Supported Cell Membrane Mimics: Neutron Reflectivity Studies of Hybrid Bilayer Membrane Structure.*

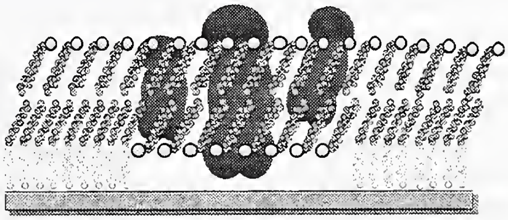
A. Plant, C. Muese, and D. Vanderah; V. Silin (Georgetown Univ.); and S. Krueger and C. Majkrzak (NIST/CNR)

Objective: Amphiphilic and hydrophobic molecules tethered to metal surfaces provide the basis for a rugged, biomimetic matrix with a structure analogous to the membrane of living cells. These model membranes provide unique opportunities for studying the structure and function of active membrane proteins because they are well suited for study with a number of emerging surface analytical techniques, including neutron reflectivity. In turn, the development of neutron reflectivity capabilities is being enhanced through the study of this rugged biomimetic experimental system.

Problem: The cell membrane is a complex arrangement of proteins, lipids and carbohydrates, and is the control point for communication between the inside and the outside of the cell. Because of the complexity of membrane proteins, and the fact that their proper structure and function requires them to be associated with an appropriate lipid matrix, there are few experimental methods that exist for studying their structure/function relations. Previous work demonstrated that the hybrid bilayer membrane (HBM) could provide a biomimetic matrix for membrane proteins and allow for their study using techniques such as vibrational spectroscopies, neutron reflectivity, surface plasmon resonance, electrochemistry, and other techniques. While the function of membrane proteins in HBMs was observed, application of neutron reflectivity to provide structural information about the location of the peptide toxin, melittin, in lipid bilayers is underway. This effort requires optimizing the composition of the stabilized membrane mimic, and improving neutron reflectivity methodology.

Approach: Hybrid bilayer membranes consist of both natural (phospholipid) and synthetic (alkanethiol) components. Long chain alkanethiols bind strongly to gold surfaces and can be exceptionally well ordered in their inter- and intramolecular interactions. The lipid components of native biological membranes, on the other hand, are relatively disordered, and have a high degree of intrachain flexibility and molecular mobility. In order to optimize the biomimetic nature of the lipid matrix of the HBMs, a series of novel compounds, thia(ethylene oxide)$_n$ alkanes, have been synthesized. The structure of monolayers of these compounds has been examined in air by infrared spectroscopy. The initial compound synthesized was composed of an ethylene oxide (EO) moiety where n equals six EO units. In collaboration with the NIST Neutron Research Facility, neutron reflectivity was used to examine the structure of HBMs containing this new chemistry and the effect of membrane protein on this structure.

Results and Future Plans: Improvements in the neutron reflectivity experiment have made unprecedented sensitivity possible. Minimizing the aqueous layer to a thickness of 15 μm, and providing a helium atmosphere around the sample reduced background neutron scattering. Reflectivity data above background have been achieved at a value of nearly 10^{-8} and a wavevector transfer of 0.5 cm^{-1}, allowing determination that the location of melittin is both the lipid headgroup region and the alkyl chain region of the lipid layer. Neutron reflectivity measurements also showed that, for n=6, the ethylene-oxide portion of the thia(ethylene oxide) alkane tether was unhydrated even in the presence of melittin. Future work involves modifying the fabrication methodology to prepare mixed monolayers of the thiahexa(ethyleneoxide)alkane plus phospholipid molecules.



Schematic of membrane proteins inserted in an engineered HBM

22. *Sum-Frequency Generation as an* In situ *Spectroscopic Probe of Hybrid Bilayer Formation*

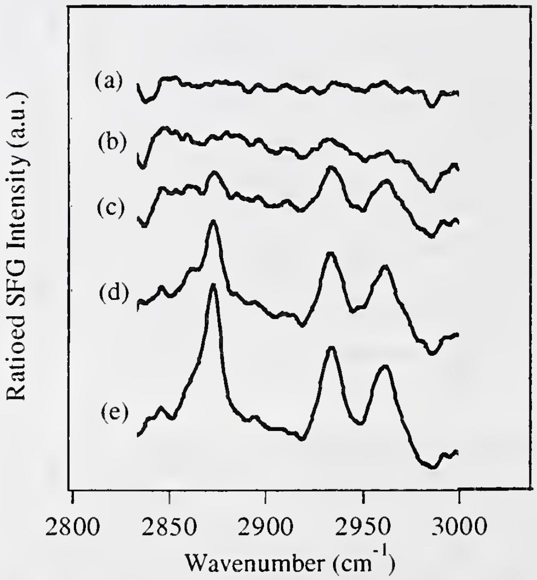
T. Petralli-Mallow, L.J. Richter (837),
K.A. Briggman (844), J.C. Stephenson (844) and
A.L. Plant

Objective: To develop vibrationally-resonant sum-frequency generation (VR-SFG) as an *in situ* probe of hybrid bilayer formation and structure at liquid/solid interfaces.

Problem: Hybrid bilayer membranes (HBM), in simplest form consisting of a phospholipid monolayer on an alkanethiol self-assembled monolayer (SAM) on gold, are promising matrices for new biomaterials. Since the mechanism of bilayer formation may affect the resulting composition of the HBM, an *in situ* spectroscopic method of following HBM formation and structure is highly desired. Sum frequency generation (SFG), a nonlinear optical vibrational spectroscopic technique, is among several methods being developed as *in situ* probes of HBMs. SFG offers powerful advantages due to its high surface selectivity, submonolayer sensitivity, and excellent spatial, spectral and temporal resolution. SFG is potentially able to give direct information about the structure, orientation, aggregation, and organization of surface-associated biomolecules.

Approach: In SFG, two laser beams of different wavelengths, one visible (V) and one infrared (IR), are simultaneously reflected from an interface. Because of the nonlinear properties of the surface, a visible photon ω_v and an infrared photon ω_{IR} can interact to create a third photon with a frequency that is at the sum of the two photon frequencies, $\omega_{SF} = \omega_v + \omega_{IR}$. Thus, the broadband sum-frequency generation system (BBSFG) developed at NIST is being utilized in these studies. In BBSFG, broad bandwidth IR is used so that an entire SFG spectrum is collected from every laser pulse, which allows rapid spectral acquisition. Vibrational spectra are routinely collected on the order of minutes, well within the time resolution needed to follow HBM formation. Because SFG band intensities may be proportional to the number of adsorbed molecules, the adsorption of phospholipids during the formation of an HBM can be followed *via* the growth of SFG bands over time.

Results and Future Plans: The figure shows SFG spectra before and throughout d13-dipalmitoylphosphatidylcholine (d13-DPPC) vesicle fusion at the buffer/deuterated octadecanethiol (d-ODT) interface. The strong features in the spectra are assigned to methyl vibrational modes: the CH_3 symmetric stretch at 2873 cm^{-1}; the CH_3 asymmetric stretch at 2961 cm^{-1}; and a Fermi resonance between the CH_3 symmetric stretch and a CH_3 bend at 2934 cm^{-1}. Methylene resonances would be expected at 2850 cm^{-1}, assigned to the CH_2 symmetric stretch, and a broad feature in the region of 2890 - 2930 cm^{-1}, assigned to a Fermi resonance between the CH_2 symmetric and the CH_2 bend overtones, and the CH_2 asymmetric stretch. The observation of no or very weak methylene resonances indicates that the acyl chains of d-13 DPPC are highly ordered and are in predominantly an *all-trans* configuration. Based on the previously established relationship between the sign of the SFG bands and a monolayer's molecular orientation on gold, the positive bands in the SFG spectra indicate that the terminal methyl groups of the acyl chains have a net polar orientation towards the d-ODT monolayer. This is consistent with the expected orientation of the phospholipid acyl chains due to van der Waals' interactions with the d-ODT alkane chains. The time frame of HBM formation observed by the SFG studies, roughly 45 minutes, is within the time



VR-SFG spectra collected at (a) 2 min., (b) 6 min. (c) 12 min. (d) 24 min. and (e) 46 min. after the introduction of vesicles to the buffer solution.

frame of HBM formation observed by surface plasmon resonance. The observation that SFG spectral features nearly disappear after an air and ethanol rinse indicates significant disruption and removal of the adsorbed phospholipid, also in agreement with SPR results. Future plans include SFG studies to molecules whose structure and properties are likely to be important to the functioning of HBM-based biosensors, such as the phospholipid gel-to-liquid condensed phase transition temperatures.

23. *Transition of the Protein Data Bank to the Research Collaboratory for Structural Bioinformatics from Brookhaven National Laboratory*

__T.N. Bhat__, P. Fagan (231), N. Thanki (NIST/UMBI), D. Hancock, M. Tung, and G. Gilliland; H. M. Berman and J. Westbrook (Rutgers Univ.); and H. Weissig and P. E. Bourne (Univ. of Cal., San Diego)

***Objective*:** To create a resource based on the most modern technology that would facilitate the use and analysis of structural data and thus create an enabling resource for biological research.

Problem: Initial use of the Protein Data Bank (PDB) had been limited to a small group of experts involved in structural research. Today depositors to the PDB have varying expertise in the techniques of X-ray crystal structure determination, NMR, cryoelectron microscopy and theoretical modeling. Users are a very diverse group of researchers in biology, chemistry, and community scientists, educators, and students at all levels. The tremendous influx of data soon to be fueled by the structural genomics initiative and the increased recognition of the value of the data toward understanding biological function, demands new ways to collect, organize, and distribute the data.

Approach: A key component of creating the public archive of information is the efficient capture and curation of the data—data processing. Data processing consists of data deposition, annotation, and validation. A fully documented and integrated data processing system has been developed and implemented. This system currently allows the deposition of structure co-ordinates from X-ray, NMR and other biophysical approaches. The PDB staff recognizes that NMR data needs a special development effort. Historically these data have been retrofitted into a PDB format defined around crystallographic information. As a first step towards improving this situation, the PDB did an extensive assessment of the current NMR holdings and presented their findings to a Task Force consisting of a cross section of NMR researchers. The PDB is working with this group, the BioMagResBank (BMRB), as well as other members of the NMR community, to develop an NMR data dictionary along with deposition and validation tools specific for NMR structures.

One of the most difficult problems that the PDB now faces is that the legacy files are not uniform. The introduction of the advanced querying capabilities of the PDB makes it critical to accelerate the data uniformity process for these data. We are now at a stage where the query capabilities surpass the quality of the underlying data. This project is being approached in two ways. Families of individual structures are being reprocessed using ADIT. The strategy of processing data files as groups of similar structures facilitates the application of biological knowledge by the annotators. In addition, we are examining particular records across all entries in the archive.

Results and Future Plans: Currently the PDB is processing and incorporating into the database 50 to 75 structures per week. The user query rate of this public resource is more than a million hits per month (more than one hit per second 24hr/7d). In the coming months, the PDB plans to continue to improve and develop all aspects of data processing. Deposition will be made easier, and annotation will be more automated. This will in turn help the data uniformity project's goal of improving the quality of the data in the archive, a major focus of the PDB.

In the data uniformity effort about one-third of the PDB entries have been processed using the file-by-file method. In the process of examining records across all entries in the archive, the R-factor and resolution parameters have been completed. These corrections have been entered into the database. The practical consequence of this is that one can now query on these parameters and produce reliable answers. Work on additional parameters including primary citation, ligands and macromolecule name and source is currently underway.

These are exciting and challenging times to be responsible for the collection, curation, and distribution of macromolecular structure data. With the advent of a number of structural genomics initiatives worldwide this number is likely to increase. It is estimated that the PDB, which currently contains more than 10,500 structures, could triple or quadruple in size over the next 5 years. This presents a challenge to both timely distribution while maintaining high quality services for the community. The PDB's approach of using modern data management practices should permit scaling to accommodate a large data influx. The maintenance and further development of the PDB are community efforts. The willingness of others to share ideas, software, and data provides a depth to the resource not obtainable otherwise.

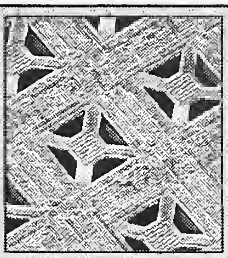
III. Process Measurements Division (836)

Gregory J. Rosasco, Chief

A. Division Overview

Mission:

The Process Measurements Division develops and provides measurement standards and services, measurement techniques, recommended practices, sensing technology, instrumentation, and mathematical models required for analysis, control, and optimization of industrial processes. The Division's research seeks fundamental understanding of, and generates key data pertinent to, chemical process technology. These efforts include the development and validation of data-predictive computational tools and correlations, computer simulations of processing operations, and provision of requisite chemical, physical, and engineering data.



Process Measurements

G. Rosasco, Chief

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Organizational Structure:

The Division, which contains the equivalent of 67 full-time employees, is organized into five groups with mission statements as follows:

Fluid Flow Group

- Establish, maintain, and disseminate the reference standards needed by U.S. industry for fluid flow rate and liquid quantity measurements,
- Conduct research to advance fluid transfer measurement science,
- Establish and maintain international comparability in fluid flow rate and fluid quantity measurements, and
- Interact with industrial counterparts and standards committees to provide expertise and assistance as requested to advance fluid transfer technology for U.S. industry and other government agencies.

Process Sensing Group

- Develop, validate, and apply state-of-the-art measurement techniques and sensors for process optimization and control of plasma reactors used in semiconductor manufacturing,
- Develop scientific and technological underpinning for application-tunable, low-cost, micro-machined gas sensor arrays to meet measurement needs in process control, emissions monitoring, and hazardous gas detection, and
- Provide scientific and technological foundation for the use of ultrathin organic films in sensing and diagnostic applications in chemical and biochemical process monitoring and health care.

Thermometry Group

- Realize, maintain, and disseminate the national standards for:
  - temperature, (the International Temperature Scale of 1990, over the range 0.65 K to 1235 K) and
  - humidity, (moisture in air: 5 nmol/mol to 75 mmol/mol),
- Perform research on developing or improving primary standards and measurements for temperature and humidity,
- Develop methods and devices to assist user groups in the assessment and enhancement of the accuracy of their measurements of temperature and humidity, and
- Co-ordinate and participate in international comparisons of realizations of the International Temperature Scale of 1990 and of national standards of humidity.

Pressure and Vacuum Group

- Develop and maintain primary pressure, vacuum, and low gas flow standards and disseminate the measurement capability to U.S. industry,
- Advance pressure, vacuum, and low gas flow measurement science:

- Conduct research to develop measurement standards and techniques to meet U. S. industry requirements.
- Perform benchmark measurements of material properties and investigate fundamental physics of industrially important phenomena which require state-of-the-art pressure, vacuum, and low flow measurements, and
- Collaborate with industry and academia in the development of new instrumentation to improve industrial process control or for use in critical scientific measurements.

Thermal and Reactive Processes Group

Develop advanced mathematical models, advanced measurement techniques, standard measurement practices and performance data for analysis, control, standardization, and optimization of key industrial processes; current focus is on:

- liquid atomization and spray combustion, and
- chemical vapor deposition.

Programs:

Current Division programs are: *Measurement Standards and Calibration Services, Measurements and Models for Semiconductor Processes, Chemical Sensors, Spray Combustion, and Optical Process Metrology.* Brief descriptions of these programs along with program highlights follow and references to selected Technical Reports are given below.

Measurement Standards and Calibration Services

Process and quality control and equity in commerce ultimately depend on the accuracy of measurements. This generally requires calibration of instruments against, or use of procedures assuring traceability to, reference standards. Therefore, measurement standards and calibration services are a very major part of the Division's activities. We provide support critical for temperature, humidity, fluid flow rate, pressure, vacuum, gaseous leak rate, liquid density and volume, and air speed measurements with almost 1000 standard tests and calibrations performed each year. The Division's commitment to provision of these services involves many facets:

- the establishment, maintenance, and improvement of the primary standards;
- continuing comparisons of these standards with those of other nations;
- development of suitable mechanisms for transferring the requisite measurement accuracy to customers in the field and in secondary calibration laboratories; and
- continual attention to calibration service efficiency and measurement quality.

The Division's comparisons of standards activities with other nations involve a number of Key Comparisons (KC) of U.S. national standards to those established and maintained by the National Measurement Institutes (NMIs) of other nations. These KCs quantify the level of equivalence among NMIs to eliminate measurement-based trade barriers. Key comparisons are organized by the respective Consultative Committees of the Committee International des Poids et Mesures (CIPM); they are initiated in selected NMIs which are then designated Pilot Laboratories. These Pilot Laboratories design and pre-test the transfer standards and test procedures; they arrange and schedule tests among participating NMIs; and they analyze data and report results.

Reports covering the efforts in these areas are presented in Technical Reports 1-9, below. Program Highlights for FY99 include the following:

<u>Comparisons of realizations of the International Temperature Scale of 1990 (ITS-90).</u> As part of an international comparison of realizations of the ITS-90, and/or of its defining fixed points, over the entire range of the scale at the highest levels of accuracy, we are participating in four Key Comparisons (KC) of realizations of the ITS-90 organized by the Committee International des Poids et Mesures (CIPM) Consultative Committee for Temperature, and in a comparison of transportable cryogenic triple-point cells organized by Physikalisch-Technische Bundesanstalt (PTB) as a EUROMET project. NIST co-ordinated, and served as the pilot laboratory for, KC 3 [83.8058 K (Ar triple point (TP)) to 933.473 K (Al freezing point (FP))], with 14 national laboratories plus Bureau International des Poids et Mesures (BIPM) participating. Also, NIST served as sub-co-ordinator for KC 4 [933.473 K to 1234.93 K (Ag FP)]. Both sets of comparisons involved circulating fixed-point cells and one or more (high-temperature) standard platinum resistance thermometers [(HT)SPRTs]. The experimental aspects of these KCs were completed in FY99. The analysis at NIST of the data from all participants of KC 3 will be completed in early FY 2000 and a report prepared. We have expanded KCs

3 and 4 to the Centro Nacional de Metrologia (CENAM) in Mexico as part of a Sistemo Interamericano Metrologia (SIM) KC that we are piloting within NORAMET. The relevant fixed-point cells and SPRTs for KC 3 were sent to CENAM in FY99.

International Comparisons of Pressure and Vacuum Standards. The Division provides national standards and calibration services for pressure and vacuum measurements over 16 decades of pressure, from about 10^{-8} to 10^{+8} Pa. As in the temperature standards area, international comparisons have grown in importance with NIST participating in six key comparisons dealing with pressure and vacuum standards. These are organized under the Consultative Committee for Mass (CCM) and Related Quantities of the CIPM. We are leading, *i.e.*, serving as the pilot laboratory, three of these comparisons. These are subatmospheric and vacuum comparisons that have required NIST to develop totally new transfer standards, instrumentation, and protocols. The two subatmospheric comparisons were formally completed in FY99, with data analysis and a draft report for participant circulation to be completed in 2000. Circulation of the transfer standard for the vacuum comparison began in the spring, with completion scheduled for the fall of 2000. With the exception of the vacuum comparison, NIST has now completed testing in all the key comparisons. Division personnel attended the 3rd International Pressure Metrology Conference of the CCM in Turin, Italy, and reviewed key comparison status at the triennial meeting of the CCM in Paris, France. Additionally, data collection for a multi-laboratory comparison for near-atmospheric pressure measurements within SIM was also completed. This involved visits by NIST staff to the participating NMIs to assist in use and operation of the transfer standards. Data analysis and a draft report for the SIM comparison will also be completed in the coming year.

International Comparisons of Fluid Transfer Standards. In the past year we have participated in a number of comparisons of our fluid quantity and flow measurement standards among other NMIs and other domestic testing laboratories and industrial facilities. Recent results include:

Air speed: NIST joined 11 other EUROMET laboratories in comparing air speed standards over the range of 0.3 m/s to 20 m/s, using vane anemometers. Results indicated that while the individual laboratories claimed expanded uncertainties of the order of 0.4 percent, the spread between the results at 20 m/s was about 6 percent. Accordingly, three actions are being planned:

1. EUROMET is planning to investigate vane-blockage effects in small tunnels,
2. Since NIST and PTB use laser Doppler anemometers (LDA) as primary standards for air speed, we are planning to compare our standards using a portable LDA as a transfer standard which will be independent of the wind tunnel size, and
3. NIST and PTB will compare standards using small-blockage, Pitot-static tubes with high precision electronic manometry as the transfer package.

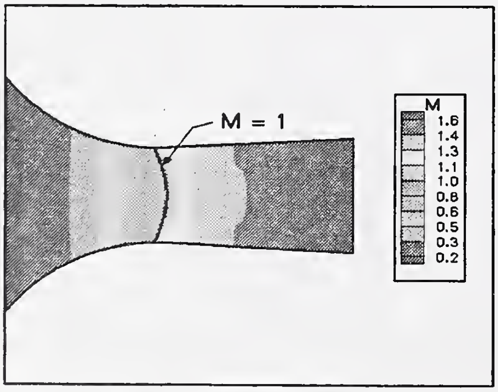
Liquid Volume: A NORAMET comparison of liquid volume standards was conducted over the range from 50 ml to 50 l. The National Research Council (NRC) Canada served as the pilot lab for the 50 ml and 100 ml measurements which were done using pycnometers with NIST and CENAM. CENAM piloted the 50 l measurements which were done using a test measure with NIST, PTB, and NRC Canada. Results for all volumes tested agreed within the stated uncertainties of the participating laboratories.

Liquid Flow: The results for the National Engineering Laboratory [NEL(UK)] initiated water flow comparison using tandem 200 mm orifice meters showed that NIST produced results that were within the claimed uncertainties of all NMI participants. The NIST tests in this program included, for the first time in any NMI test, the real-time monitoring of the pipe flow profile that entered the transfer standard while it was being tested. This was done using our non-intrusive, eight path, travel-time ultrasonic flow metering and diagnostics unit upstream of the transfer standard. This enabled complete assessment of our flow standards capabilities in an actual international comparison, and it should set a proper precedent for future tests of this kind. In addition to NEL(UK) and NIST, other NMIs included the National Research Laboratory of Metrology(NRLM) in Japan, CENAM, and the Delft Hydraulic Laboratory (the NMI for water flow measurement in the Netherlands). An additional participant was Alden Research Laboratories (in the U.S.). This program is planned by NEL(UK) to evolve into a EUROMET comparison.

Gas Flow: We have been involved in three comparisons of our gas flow standards using critical flow venturis (CFVs) as transfer standards:

1. CENAM served as the pilot laboratory for a NORAMET comparison with NIST in which results agree within 0.23%, which is within the uncertainties claimed,
2. A Ford Motor Co. comparison which involved NIST four years ago and which has been done again in 1999 shows NIST results reproducing to within 0.05%. This testing program includes NMIs in England, Japan, Korea, and Taiwan. It also includes the Colorado Engineering Experiment Station, Inc. (CEESI) and a range of Ford labs in the U.S. and abroad. The level of agreement was generally better than 0.1% and the largest difference between NIST and any other NMI is 0.25%, and
3. We have also just finished the measurements for a French-initiated EUROMET comparison involving 11 participants that include NMIs in Germany, UK, Denmark, Norway, Switzerland, and the Netherlands as well as other labs in France and Canada and CEESI and the Southwest Research Institute (SWRI) in the U.S. Preliminary results show the agreement is within 0.2%.

This year our gas flow metering research efforts yielded results that for the first time explain the abnormal behavior seen in small critical nozzles while flowing certain gas species (*e.g.*, CO_2). Traditionally, the discharge coefficients of critical nozzles have been assumed to be gas species independent. However, experiments with small nozzles have shown that gas species effects can yield errors of up to 3%. Our CFD research results, see figure, indicated that vibrational relaxation effects account for the errors observed. Once these effects are considered, numerical predictions agree with experimental results at the 0.4% level for all gas species. Further details are given in Technical Report 8, below.



Mach Number, M, Distribution in a Critical Nozzle

Chairmanship of the Working Group for Flow, CCM: In the past year, an Ad Hoc Group for Flow (AHGF) was formed to assess the international flow community for its interest in forming a Working Group for Flow (WGF) in the CCM. The objective of the WGF is to arrange and conduct Key Comparisons of flow standards among the NMIs to establish the equivalencies of these standards. The AHGF assessment indicated significant world-wide interest in forming the WGF, and, at the recent CIPM meeting in Paris, the formation was made official, and Dr. George E. Mattingly was named its Chairman. The elevation of the area of flow to the status of a working group of a consultative committee of the CIPM highlights the importance of this area of metrology to international commerce.

Measurements and Models for Semiconductor Processes

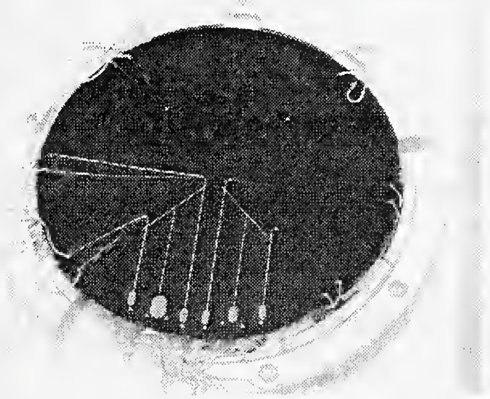
While our measurement and calibration services are provided to a wide range of customers, we also have measurement support programs focused on specific strategic technologies, such as maintaining U.S. leadership in semiconductor device manufacturing. As part of NIST's National Semiconductor Metrology Program (NSMP), the Division is selecting, developing, evaluating, and validating process measurement technologies important in semiconductor manufacturing. These efforts include:

- provision of improved thermocouples for control of thermal processing equipment, including rapid thermal processing (RTP) systems,
- low-range gas flow standards,
- evaluations and models of the performance of residual gas analyzers used to monitor gaseous composition in fabrication tools,
- models for contamination control in thermal CVD processes,
- methods to determine electrical, physical, and chemical properties of plasmas used for etching and deposition processes, and
- very low-level water vapor measurements for contamination control in process gases.

In some of these efforts, we make use of a reference processing reactor prototypical of industrial manufacturing. This allows critical tests of the measurement approach and its utility for the intended application. Because processing systems are complex, with strongly coupled chemistry and mass-transport and, in the case of plasma reactors, complex electrical interactions, reference reactors are subject to extensive modeling and validation efforts as an integral part of the measurement support activity. These models and supporting data play a critical role in the Semiconductor Industry Association's (SIA) National Technology Roadmap for Semiconductors (NTRS). In fact, modeling is specifically identified not only as a "crosscutting technology," but as "pervading all crosscuts." Our program in this area, partially supported by the NSMP, seeks to develop and validate benchmark chemical mechanisms and supporting thermochemical and kinetic data, for equipment and process design and control.

Our research in this program is described in Technical Reports 10-13, below. Program highlights for FY99 include the following:

Improvement in accuracy of surface temperature measurements in rapid thermal processing (RTP) of semiconductors. The semiconductor manufacturing industry needs higher accuracy than currently available in measuring the temperature of silicon wafers during processing to achieve goals in product quality and device performance. Consequently, the industry roadmap now requires an uncertainty of ≤ 2 °C at 1000 °C for RTP for the next generation of wafer patterning. Radiation thermometers are used in RTP but the uncertainty in measurements made with them is unacceptably large when the thermometers are calibrated against blackbodies.

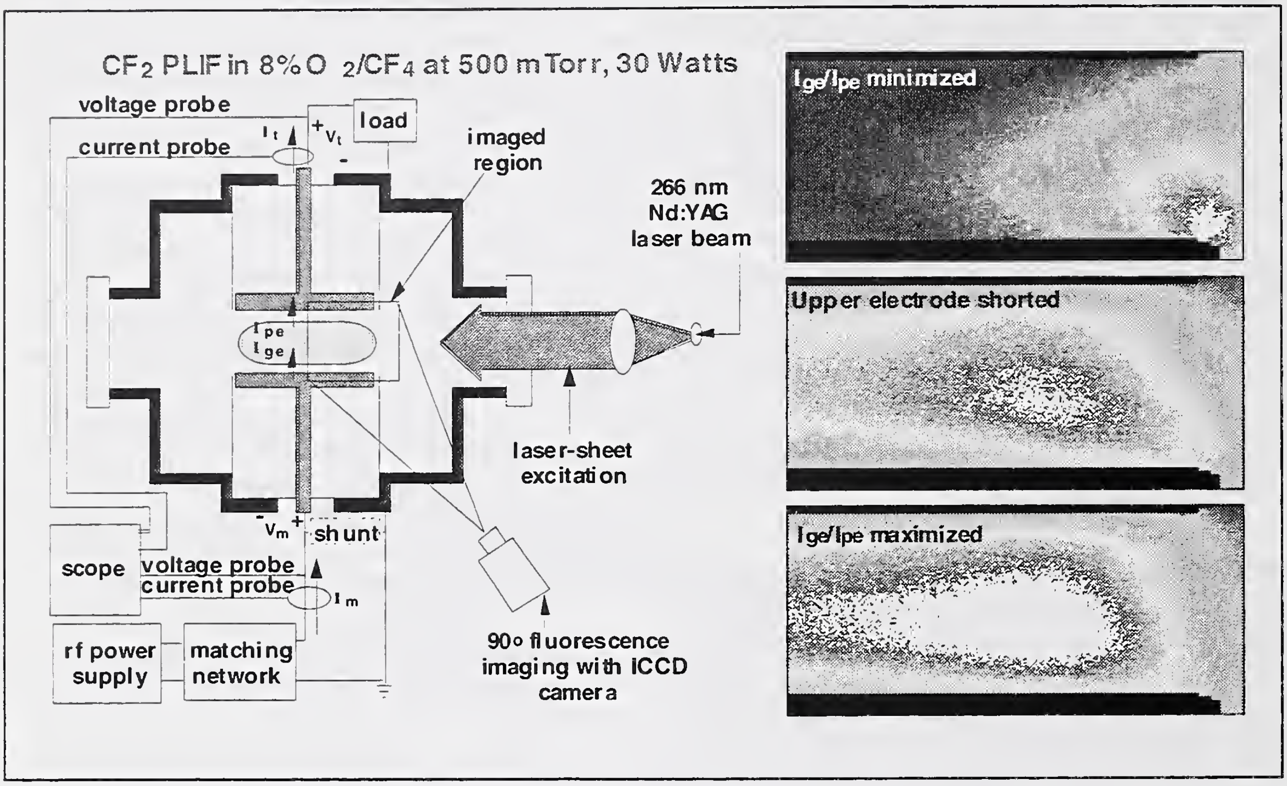


Thermocouple instrumented calibration wafer, 203 mm diameter.

We are investigating the calibration of light-pipe radiation thermometers (LPRTs) using Si wafers instrumented with combinations of stable thin-film and Pt/Pd wire thermocouples (TCs) such as shown in the figure above. The thin-film TCs minimize errors from heat transfer that would be present for other types of temperature sensors. We have used our TC-instrumented calibration wafers in the NIST RTP tool for calibrating radiometers up to 900 °C. Using this technique, the combined uncertainty was reduced to about 2 °C. During FY 2000 we will extend the measurements in the RTP tool to 1000 °C and also evaluate the effect of various wafer emissivities on the calibration. Additionally, we will investigate the effect of the temperature of the LPRT itself on the temperature it measures to better determine the proper procedure for its calibration. As part of a CRADA with SEMATECH and as a step in transferring our technology to the commercial sector, we have designed, fabricated, tested and delivered TC-instrumented calibration wafers to SEMATECH for their evaluation in their RTP tool.

Electrical Control of Plasma Spatial Uniformity. The Division has demonstrated a new method for controlling the spatial distribution of reactive chemical species in fluorinated gas plasmas. Such plasmas are widely used by the semiconductor industry to etch silicon, silicon dioxide, and silicon nitride films and to clean the reactors that deposit these films.

The method was demonstrated in CF_4/O_2 and C_2F_6/O_2 chamber-cleaning plasmas, in a plasma reactor with two electrodes. Normally radio-frequency power is applied to one electrode while grounding the opposite reactor electrode. To implement the new control method, a variable-impedance electrical load replaced the ground connection of the second electrode. The load controls whether the radio-frequency current injected at the powered electrode flows to the second electrode or to other surfaces inside the reactor. By altering the flow of current through the plasma, we are able to control where plasma electrons are heated and reactive species are generated. This in turn allows control of the spatial distribution of reactive species, as verified by two-dimensional broadband optical emission measurements and two-dimensional planar laser-induced fluorescence of the CF_2 radical, as shown in the figure on the next page. By varying the load impedance, we obtain greater control over the spatial distribution of reactive species, beyond what can be obtained by only varying the pressure, power, gas mixture, or flow rate. This control method could potentially be used to direct reactive species in chamber-cleaning plasmas toward the reactor surfaces most in need of cleaning, or to increase the uniformity of reactive species across the wafer surface during etching.



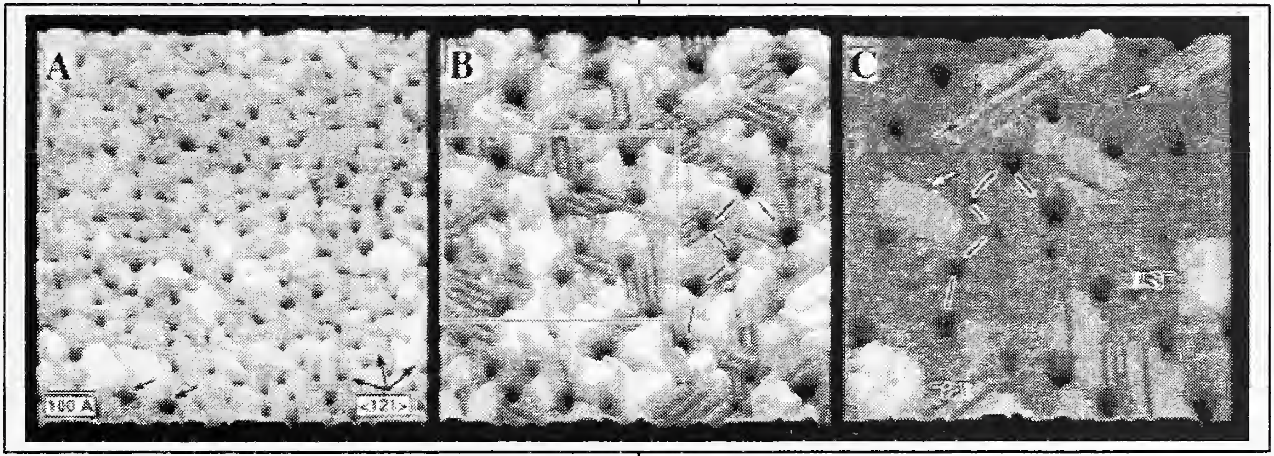
Chemical Sensors

The Division has a significant effort in chemical sensor technology with two research activities: micro-machined gas sensor arrays and diagnostic applications of self-assembled monolayers (SAMs). The first activity is collaborative with the Semiconductor Electronics Division of the Electronics and Electrical Engineering Laboratory. The technology is based on NIST developed, and patented, 'micro-hotplate' arrays formed by silicon micro-machining. Chemical sensors are fabricated by depositing metal oxides, *e.g.*, SnO_2, and surface-dispersed catalytic metal-additives on the micro-hotplate to form robust, electrical-conductance-based sensing elements. The objectives of our effort are to develop the knowledge base required to optimize multi-species detection and quantitative analysis and to resolve generic device-processing issues that could limit commercial application. The second activity investigates alkanethiol monolayers, of the general formula $X(CH_2)_nSH$, self-assembled on the surfaces of noble metal substrates. These SAMs are robust, reproducibly prepared structures with highly tunable surface properties, and serve as a model system for the study of many sensing applications. An example is DNA microchip technology, which has potential application in the areas of disease detection, toxicology, forensics, industrial processing, and environmental monitoring. Our research currently is focused on the self-assembly of DNA monolayer films on surfaces, with the goal to understand how the molecular surface structure of the DNA probes impacts the performance of the microchip devices.

The research in Chemical Sensors is detailed in Technical Reports 14-16, below. Program highlights for FY99 include:

Molecular Resolution Snapshots of Alkanethiol Monolayer Degradation. Until recently, self-assembled monolayers were thought to be stable in an ordinary lab environment. However, studies have shown that ozone in the air can degrade the self-assembled monolayer film by oxidizing the thiol headgroup of the alkanethiol molecules. The Process Sensing Group examined the time evolution of this process by exposing monolayers to increasing doses of pure ozone while recording STM and photoemission data. The photoemission results show that ozone incorporates oxygen into the monolayer by oxidizing the thiol terminus forming a sulfinate or sulfonate.

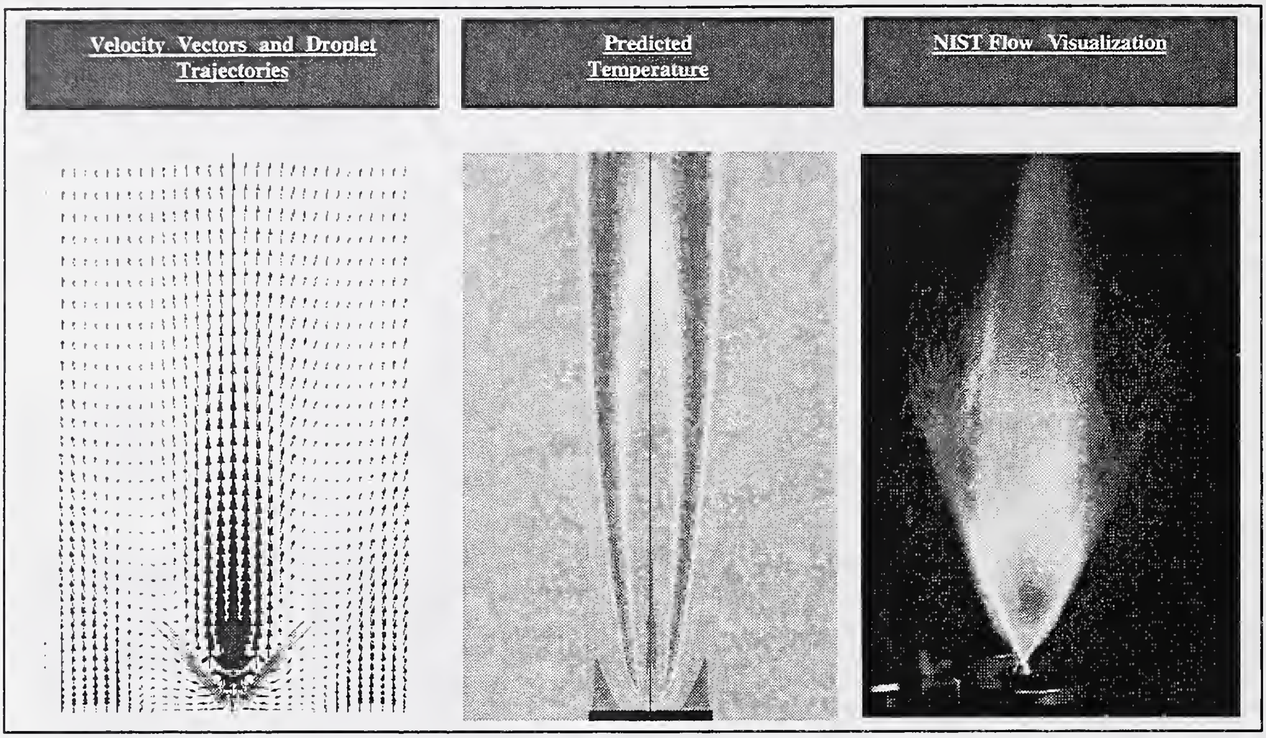
The topographic data, a subset of which is shown in the figure below ozone attacks the crystalline monolayers preferentially at the network of domain boundaries between the molecules (dark crevices in frame A). As the ozone exposure increases, the reaction spreads into the crystalline domains converting them to a different crystalline phase (striped features in frame B). The topographs provided evidence of an unexpected result: as the reaction front propagates, the crystalline monolayer melts and forms either a 2-dimensional liquid or a 2-dimensional amorphous solid (disordered regions in frame C). The results demonstrate that crystallographic defects in monolayer films can play an important role in their chemical reactions. Insight garnered from the study points to possible strategies to improve the monolayer's stability in ozone, such as decreasing the density of domain boundaries or decorating the boundaries with molecules that are inert to ozone.



Spray Combustion

Addressing industrial problems associated with the operation and design of thermal reactors is the main focus of the Division's other programs in process technology. Based upon a unique combination of spray measurement capabilities and a laboratory-scale prototype spray combustion facility, the spray combustion program emphasizes measurement of the role of droplet dynamics (generation, transport, and mixing) in determining the performance (efficiency and emissions) of spray-based energy production and waste destruction systems. The program's primary objective is provision of benchmark data to validate computational models and to establish a means to correlate performance with operating conditions. Ties to the modeling community are being strengthened to provide industrial users with well-validated spray combustion simulation capability. See simulations in the figure for droplet/gas velocities (left) and gas temperature (center), and NIST flame (right), courtesy Computational Fluid Dynamics Research Corporation. The work in this area is described in Technical Report 17. Program highlights for FY99 include:

Benchmark Database for Multiphase Combustion Models**.** This year an alpha version of the benchmark multiphase-combustion database was released to industrial and academic collaborators for their use in validating multiphase combustion models and submodels. In support of this effort, all potential collaborators were invited to a workshop at NIST. The focus of the workshop was to familiarize the participants with NIST's reference spray combustion facility, assess the information currently provided in the database, review preliminary findings from simulations of the NIST facility, allow modelers to express their data needs, and provide an opportunity for feedback concerning future measurements. Attendees at the workshop represented industry (chemical, power, energy, and software developers), other government agencies, and academia. Discussions focused on the most critical industrial needs and how NIST can provide the greatest impact.


Velocity Vectors and Droplet Trajectories

Predicted Temperature

NIST Flow Visualization

Optical Process Metrology

One element of the Division program is being conducted jointly with the Analytical Chemistry and the Surface and Microanalysis Science Divisions. The program seeks to develop frequency and intensity standards for Raman spectroscopy. While recent breakthroughs in instrumentation hold promise for very widespread applications of Raman analyses in industry, the lack of these standards is a major barrier to its full deployment. A second major effort in this area seeks to exploit the technique of cavity-ring-down spectroscopy (CRDS) to enable extremely high sensitivity, quantitative measurements of gas phase and surface-adsorbed species. Primary applications in the gas phase involve the development of next generation, potentially primary, moisture and partial pressure analyzers. The extension of cavity-ring-down principles to surface adsorbed species relies on another NIST invention, evanescent wave CRDS.

Some of these research results are documented in Technical Reports 18 and 19. Program highlights for FY99 include:

Glass Artifact Standards for Raman Spectroscopy. In the project to develop standards for Raman spectroscopy, significant progress was made this year, in collaboration with the Surface and Microanalysis Science Division and Analytical Chemistry Division, to determine the suitability of specially tailored fluorescent glasses as an artifact standard for Raman spectroscopy. Three glass compositions, tailored for each of three laser wavelengths and Raman regimes (*i.e.*, 514.5 nm, 785 nm, and 1064 nm) were found to provide featureless broadband fluorescent emissions that were resistant to photobleaching upon laser irradiation, and are ideally suited as secondary luminescence standards. Measurements with several different spectrometers are currently underway at NIST to determine the relative irradiance of these glasses. These studies will provide the basis for round robin measurements through ASTM E13.08 to determine intensity-corrected Raman spectra employing these glasses.

Optical Measurement of Water Partial Pressure via Cavity-Ring-Down. Conventional wisdom among practitioners of cavity ring down spectroscopy holds that maximum sensitivity comes from building the longest cavity practicable, in many cases meters-long. However, for pulsed excitation of these long cavities, light wave interactions inside the cavity should give rise to highly complicated and irreproducible decay signals. However, the theory of CRDS, recently developed in our research, suggests that through the excitation of a single optical resonance in a short cavity, the effects of this "mode beating" would be eliminated. This conjecture was validated by experiments with a 10-cm cavity and demonstrated more than a 100-fold sensitivity increase over our previous results using a 1-m cavity. Moreover, the relative standard deviation of ring-down times from an ensemble of measurements agreed with that estimated from single resonance measurements, indicating that drift has virtually been eliminated. A measurement imprecision of ~0.1% during the course of a day's measurements and ~0.3% over many days was also found. This level of imprecision should put detection sensitivities very close to the SIA Roadmap year 2001 target for water measurements, a fact that has been confirmed in very recent experiments.

Awards in FY99:

- **Dr. Gregory J. Rosasco** was awarded the Department of Commerce's Silver Medal in the leadership category in recognition of his role in establishing standards and services in the Process Measurements Division which meet industry's needs and are recognized for their outstanding quality worldwide.

- **Drs. Joseph T. Hodges, J. Patrick Looney, and Roger D. van Zee** received the Department of Commerce Bronze Medal for their research that produced fundamental advances in the theory and practice of Cavity Ring Down Spectroscopy.

- **Dr. Pedro I. Espina** received the "Best Paper" award at the 1999 Measurement Science Conference for his paper entitled *"Tele-Calibration of Gas Flow Meters."*

B. Selected Technical Reports

1. *Calibration and Test Services Provided by the Process Measurements Division*

G.J. Rosasco, *V.E. Bean, P. I. Espina, J. T. Hodges, A. Lee, J. P. Looney, B. W. Mangum, G. E. Mattingly, A. P. Miiller, and J. W. Schmidt.*

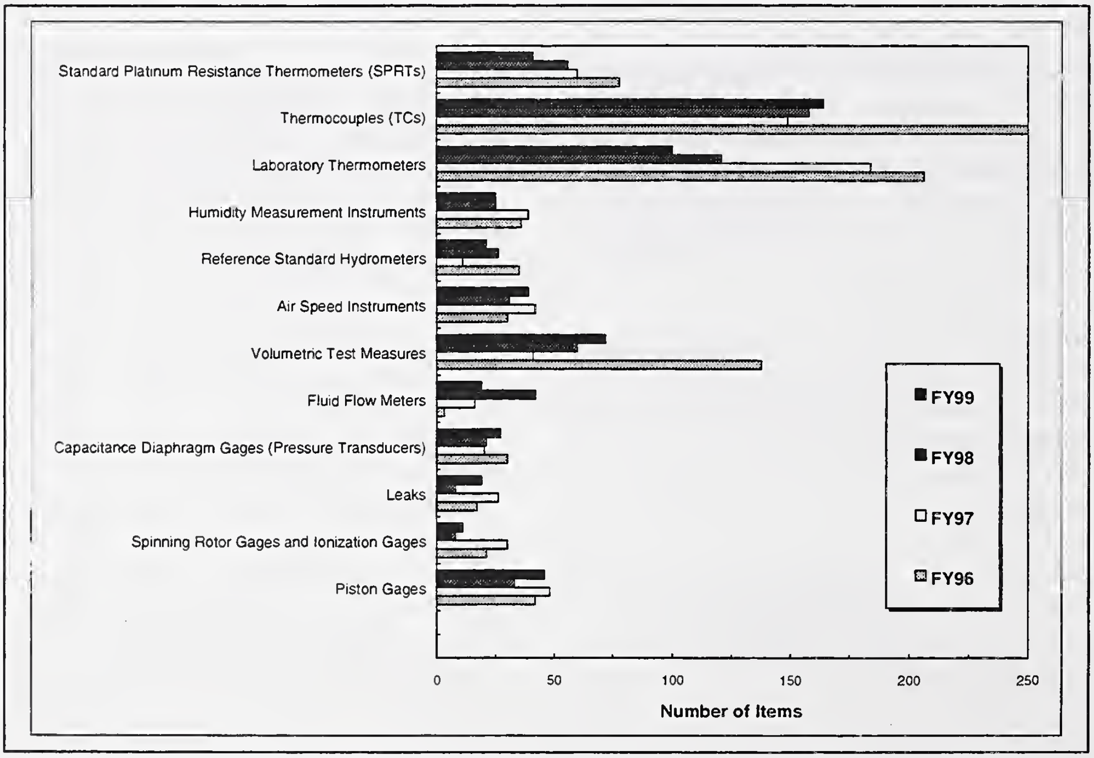
Objective: To realize and maintain national standards according to the definitions of the relevant units, and to disseminate the NIST-realized units to industry and government agencies (federal and state governments) that require calibrations against, and direct traceability to, national standards.

Problem: The Process Measurements Division is responsible for realizing, maintaining, and disseminating the national standards for measurement of temperature (in the range of 0.65 K to 1235 K), pressure, vacuum, gaseous leak rate, humidity, fluid flow rate, liquid volume and density, and air speed. Issues of primary importance in this area involve:

- assessing and meeting, to the extent practicable, customer requirements in terms of types and ranges of service and levels of uncertainty,
- improving the efficiency of calibration services,
- developing methods to enable appropriate realization of standards in the customer's laboratories, and
- serving as the primary resource supporting the national measurement system.

Approach: Provision of customer-appropriate access to national standards of measurement involves a range of activities: maintenance and improvement of primary standards, participation and leadership in U.S. and international standards activities, performance of instrument calibrations and tests, operation of Measurement Assurance Programs, proficiency and round-robin tests, development of mechanisms for realization of secondary standards in customer laboratories, and a wide-range of consultation and customer assistance services. The calibration and test services provided by the Division are described in NIST SP 250, NIST Calibration Services Users Guide, and its supplements.

Results and Future Plans: The chart below summarizes the level of activity in each service area over the past four years.



Large fluctuations, year to year, in numbers of items are often encountered; however, the total calibration workload typically varies by less than 20%. Our capabilities, experimental techniques and the facilities used to provide these services are continually upgraded, with concomitant improvements in efficiency and in measurement uncertainties. Improvements of standards and highlights of activities for temperature, humidity, and gas flows are discussed in separate reports below. Also presented in separate reports are descriptions of our extensive involvement in international comparisons, a critical element in maintaining the position of the Nation's measurement system in the world.

During the past year, we have implemented the NIST-generated, web-based Information System to Support Calibrations (ISSC). This system significantly improves our abilities to monitor progress and assess results in our numerous and diverse calibration services. It also enables efficient archiving of the data for the instruments calibrated and the efficient inclusion of these results in future reports of calibration to show instrument owners their historical records and to quantify the temporal stability of the uncertainties claimed. The ISSC also enables our calibration customers to access the status of their calibrations through password-protected security. The ISSC addresses a number of issues identified as a result of a study conducted in FY99 of the turn-around time performance for the Division's calibration services. This study examined FY97-98 performance in terms of how well we met customer expectations relative to promised completion dates for calibrations. The results of this study were presented to the Board of Directors of the National Conference of Standards Laboratories and to the Annual Workshop and Symposium of that organization. While our performance was generally good, there were areas where increased attention to meting deadlines was needed and where the automated report generation and progress tracking features of the ISSC will aid timely completion of work. The FY99 turn-around time performance showed marked improvement in all areas where problems had been identified in our retrospective study of FY97-98.

This past year, we completely re-vamped the primary high vacuum standard to eliminate intermittent leaks, reduce background pressure, significantly improve accessibility and serviceability, and installed sophisticated interlocks which prevent compromise of high vacuum in the event of unforeseen power or pump problems. In addition, a new primary flowmeter, which is central to every vacuum calibration service, was designed to extend the lower limit of measurable gas flows and reduce uncertainties. This new flowmeter will be assembled, tested and put into service in early FY 2000.

In the pressure area, we are the pilot laboratory for an 18-participant, star-pattern, pressure measurement round robin for the NCSL, which uses 200 psi (~1.4 MPa) pressure transducers as the transfer standard. Participants are public and private U.S. calibration laboratories. The round robin began in late FY98, all testing is nearly complete, and will conclude in FY 2000, with results to be presented at the 2000 NCSL Conference in Toronto.

We have also conducted for the first time a special liquid flow measurement proficiency test to establish direct NIST traceability for a U.S. manufacturer of large flow meters for variable viscosity oils. This was done using our eight path, travel-time, ultrasonic flow transfer standard and Reynolds number scaling principles. The diagnostic capabilities of this transfer standard enabled concurrent assessment of the pipe flow profiles in the manufacturer's lab as we quantified the performance of their flow standards relative to NIST standards. These precedent setting results are providing this manufacturer's bases for their product specifications claimed for future national and international sales. In addition, we conducted a special test of a prototype Doppler ultrasonic flow meter that uses an echography technique to measure the velocity profile in pipe flows and then performs velocity-area integration to determine the bulk flow; results showed this method also offers potential to improve flow measurements and standards. All of these results reinforce our plans to evolve ultrasonic techniques into primary flow standards; this will greatly facilitate the expansion of our capabilities to provide flow traceability for the full range of flow needed by U.S. industry.

Publications:

Bean, V.E. and Hall, J. M., ***"New Primary Standards for Air Speed Measurement at NIST,"*** Proc. of the National Conference of Standards Laboratories Symposium, July 11-15, 1999, Charlotte, NC, pp. 413-442.

Ellefson, R.E., and Miiller, A.P., *"Recommended Practice for Calibrating Vacuum Gauges of the Thermal Conductivity type,"* J. Vac. Sci. Techn. A, in press.

Hall, J.M., Bean, V. E., and Mattingly, G.E., *"Preliminary Results from Interlaboratory Comparisons of Air Speed Measurements Between 0.3 m/s and 15 m/s,"* Proc. of the National Conference of Standards Laboratories Symposium, July 11-15, 1999, Charlotte, NC, pp. 423-435.

Mattingly, G. E. and Yeh, T. T., *"NIST's Ultrasonic Technology Assessment Program to Improve Flow Measurements,"* NIST Tech Note 1429, Oct, 1999.

Miiller, A. P., *"Measurement Performance of High-accuracy Low-pressure Transducers,"* Metrologia, in press.

Miiller, A. P., *"Measurement Performance of High-Accuracy Low-Pressure Transducers,"* Proc. of the 3rd CCM International Conference on Pressure Metrology, Torino, Italy (1999).

Miiller, A.P., *"Report on Two International Key Comparisons: 1. Absolute Pressure (1 Pa to 1 kPa) 2. Differential Pressure (1 Pa to 1 kPa),"* CCM Low-Pressure Working Group Meeting, Sevres, France.

Nakao, S., Wright, J. D., Barbe, J., Niederhauser, B., Quintilii, M., and Knopf, D., *" Intercomparison Tests of the NRLM Transfer Standard with the Primary Standards of NIST, BNM-LNE, OFMET, and PTB for Small Mass Flow Rates of Nitrogen Gas,"* Metrologie '99 Conference, France, 1999.

Rosasco, G. J. *"A Study of Turn-Around Time for Selected Thermodynamic, Dimensional, and Mechanical Calibrations,"* Proc. of the 1999 NCSL Workshop and Symposium, pp. 1081-1094, (1999).

Schmidt, J.W., Cen, Y, Driver, R.G., Bowers, W.J., Houck, J.C., Tison, S.A., and Ehrlich, C.D., *"A Primary Pressure Standard at 100 kPa,"* Proc. of the 3rd CCM International Conference on Pressure Metrology; Torino, Italy (1999).

Schmidt, J.W., Tison, S.A., and Ehrlich, C.D. "*A Model for Drag Forces in the Crevice of Piston Gauges in the Viscous and Molecular Flow Regimes*," Proc. of the 3rd CCM International Conference on Pressure Metrology; Torino, Italy (1999).

Wright, J. D., *"Validating Uncertainty Analyses for Gas Flow Standards Via Intra- and Inter-Laboratory Comparisons,"* Proc. of the National Conference of Standards Laboratories Symposium, July 11-15, 1999, Charlotte, NC, pp.711-724.

Yeh, T.T. and Mattingly, G. E., *"Ultrasonic Technology: Prospects for Improving Flow Measurements and Standards,"* 4th Int'l Symp. For Fluid Flow Measurement, Denver, CO, June 27-30, 1999.

2. *Maintenance and Dissemination of the International Temperature Scale of 1990*

B.W. Mangum, C.W. Meyer, W.L. Tew, G.F. Strouse, G.T. Furukawa (Guest Researcher) and W.C. Ausherman

Objective: Maintain the ITS-90 as it is defined over the range from 0.65 K to 1235 K, investigate its non-uniqueness over this temperature range, and disseminate it to the user community.

Problem: The uncertainties resulting from the non-uniqueness of the scale over the range from 13.8033 K to 1243.93 K must be quantified to determined the total uncertainties in our calibrations. Also, better methods for dissemination of the ITS-90 are needed by users requiring high accuracy.

Approach: The non-uniqueness of the ITS-90 will be determined in the various temperature ranges from 13.8033 K to 1234.93 K. The ITS-90 is disseminated through calibrations, SRM fixed-point cells of pure materials, SRMs of pure metals of the defining fixed-point materials, and SRM thermometers.

Results and Future Plans: The non-uniqueness in the range from 13.8033 K to 24.5561 K, arising from the use of SPRTs and an interpolating constant-volume gas thermometer (ICVGT), was determined to have a maximum value of about 1 mK. The non-uniqueness in the ranges 273.16 K to 933.473 K and 933.473 K to 1234.93 K resulting from use of SPRTs has not been completed but efforts to determine it were continued this year. Comparison measurements on NIST HTSPRTs that had been selected for investigation on the basis of their stability upon high-temperature exposure and that had been calibrated from 273.16 K to 1234.93 K were continued in the range from 933 K to 1235 K in our high-temperature comparator. All measurements obtained to date indicate a non-uniqueness of about 2 mK, significantly smaller than that observed at other NMIs. During FY 2000, the investigation of all of NIST's HTSPRTs in this range is expected to be completed. The investigation of the non-uniqueness in the range 273.16 K to 933.473 K is expected to begin in FY 2000.

In recent years, we have had a significant effort in developing fixed-point cells, and in certifying thermometers and high-purity metals (for use in fixed-point cells), with the goal of disseminating the ITS-90, or of providing materials for use in its realization, to calibration laboratories and others requiring high accuracy. In this effort, work on the certification of 99.999 995+ % pure Ga for SRM 1751, the Ga melting-point (MP) standard, was completed in FY99. Also, work on another new SRM (SRM 1750), calibrated capsule SPRTs, covering the range from 13.8033 K to 429.7485 K, was completed in FY99. The observed deviations, ΔW, from the ITS-90 reference function were highly correlated throughout the temperature range, as illustrated in Figure 1, in the case of the Ga MP and Hg triple point (TP), indicating a high sample-to-sample uniformity within the SRM. These SRMs add to our other thermometry-related SRMs that aid in the dissemination of the ITS-90 to those requiring high accuracy (see Figure 2).

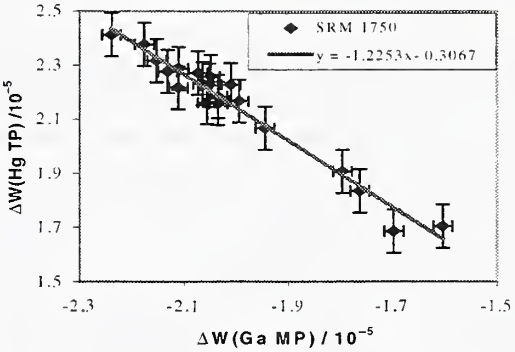


Figure 1. The deviations for SRM 1750 at the Hg TP versus those at the Ga MP

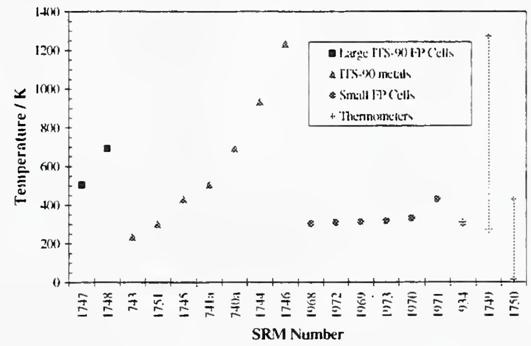


Figure 2. Thermometric SRMs

Publication:

Meyer, C.W., Strouse, G.F. and Tew, W.L., "***Non-uniqueness of the ITS-90 from 13.8033 K to 24.5561 K,***" Proc. of TEMPMEKO 99, in press.

3. *Thermodynamic Temperature Measurements*

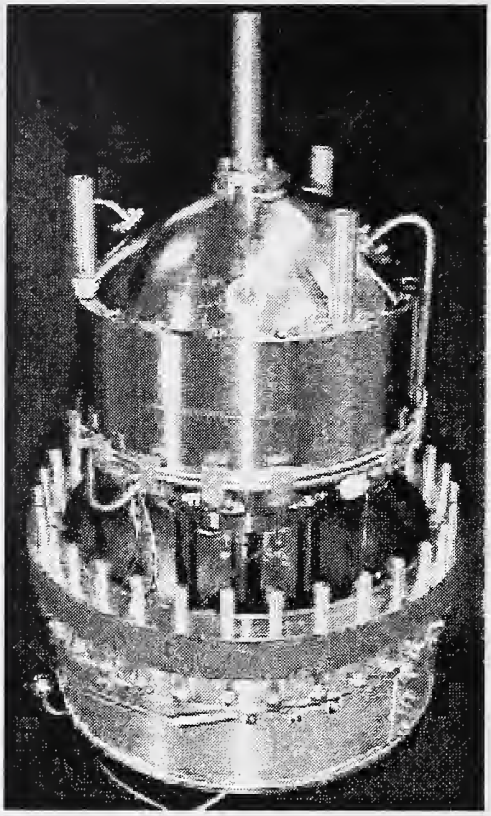
D. C. Ripple, B. W. Mangum, D. DeFibaugh (838), and M. Moldover (838)

Objective: To improve the accuracy of thermodynamic temperature measurements above 500 K by a factor of 3 to 8.

Problem: There remain unresolved inconsistencies in previous measurements of thermodynamic temperatures at 500 K and above by two groups of researchers at NIST that used different gas thermometers. Those inconsistencies led to unnecessarily-large uncertainties in the thermodynamic values assigned to the defining fixed points of the ITS-90 and, hence, to all temperatures measured on the ITS-90 by radiation techniques, for which reference is made to either the freezing-point temperature of Ag, Au or Cu.

Approach: The speed of sound of a monatomic gas is simply related to the thermodynamic temperature. Utilizing this relationship and acoustic resonance techniques, we will determine thermodynamic temperatures between 300 K and 800 K from measurements of the speed of sound of Ar in a spherical cavity. At NIST, the Boltzmann constant has been determined with such techniques with unprecedented accuracy. Acoustic thermometry up to 800 K requires development of novel transducers, control of gas impurities, and novel flow-control techniques. With a thermometer incorporating these improvements, we expect reductions in the uncertainty of the thermodynamic temperature of various high-temperature fixed points by factors of 3 to 8.

The goal for the Au point is a reduction by a factor of five to an uncertainty of 10 mK. The results will have application to radiometric measurements of temperature to much higher temperatures (upward to maximum measured temperatures of about 4000 K) since the new, more accurate values of the thermodynamic temperatures will be the basis for the radiometric measurements.



The resonator resting inside half of a spherical 3 L pressure vessel and furnace shell

Results and Future Plans: In FY99, the acoustic resonator (see Figure), its associated transducers, the pressure vessel surrounding the resonator, and the innermost furnace shell were assembled, integrated into the gas handling system, and tested. Microwave and acoustic resonances were successfully measured over the full frequency range needed for our experiments. Problems with parasitic mechanical resonances of the apparatus were successfully diagnosed, and methods of damping these resonances in an all-metal system were developed. Temperature control of the furnace to within 0.5 mK was achieved. Acoustic resonances were measured at 400 K under conditions of active thermal control and continuous gas flow, demonstrating that the thermometer is fully functional at temperatures beyond the limits of previous high-accuracy acoustic thermometers.

During FY 2000, we will continue to test the performance of the acoustic thermometer under conditions of flowing gas and active thermal control, at temperatures up to 550 K. Determinations of thermodynamic temperature will commence following completion of the furnace and testing for thermal uniformity.

4. *International Comparisons of Temperature Standards and Scale Realizations*

B.W. Mangum, G.F. Strouse, W.L. Tew, C.W. Meyer, and G.T. Furukawa (Guest Researcher)

Objective: Serve as co-ordinator for, and/or participate in, comparisons of national realizations of the ITS-90 and of some transportable cells of the defining fixed points of the scale.

Problem: Although there have been some bilateral as well as some multi-national comparisons of fixed-point cells of some defining fixed points, there have been no general comparisons of realizations of the ITS-90 or of the defining fixed points over the entire range of the scale at the highest levels of accuracy. Results of such comparisons are needed in order to develop a basis for bilateral agreements between the U.S. and other countries on the realizations of the Kelvin (through the ITS-90) and of the other SI units. Such agreements are necessary for trade purposes.

Approach: We are participating in four key comparisons of realizations of the ITS-90 organized by the CCT, and in a comparison of transportable cryogenic triple-point cells organized by PTB for EUROMET. NIST co-ordinated key comparison (KC) 3 [83.8058 K (Ar triple point (TP)) to 933.473 K (Al freezing point (FP))], with 14 national laboratories plus BIPM participating. NML and PTB served as our sub-co-ordinators for some of the laboratories. Also, NIST served as sub-co-ordinator for KC 4 [933.473 K to 1234.93 K (Ag FP)]. Both sets of comparisons involved circulating some fixed-point cells and one or more (HT)SPRTs. We are participating also in KC 1 (0.65 K to 24.5561 K) involving RIRTs, and KC 2 (13.8033 K to 273.16 K) involving capsule SPRTs (CSPRTs). We have expanded KCs 3 and 4 to CENAM as part of a NORAMET project, with NIST serving as co-ordinator. Also, we will participate in a SIM project on comparisons of various types of thermometers.

Results and Future Plans: The experimental aspects of KCs 3 and 4 were completed in FY99. Figs. 1 and 2 show the laboratories participating. The analysis at NIST of the data from all participants of KC 3 will be completed in early FY 2000 and a report prepared for the CCT. This report will be circulated among the participants and then submitted to the CCT before the meeting in January 2000 at NIST of the CCT Working Group 3 and the coordinating laboratories for all the KCs. A compilation of the data of KC 4 that we acquired as sub-co-ordinator for PTB was submitted to PTB near the end of FY99.

After measurements are completed on our two CSPRTs at NRC (co-ordinator), follow-up measurements will be made at NIST. Our two RIRTs sent to NPL (co-ordinator) for participation in KC 1 have been measured there and returned to NIST for follow-up measurements. TP cells of O_2, Ar and Ne were sent to PTB in FY99. Equilibrium H_2 and Hg TP cells will be sent in FY 2000. Since the NIST O_2 cell contains pure O_2 prepared from decomposition of $KMnO_4$, its comparison with those of the European laboratories that use commercially-available, O_2 as their source will be interesting and important.

We began the NORAMET project in FY99, with NIST providing the relevant fixed-point cells and SPRTs to CENAM. Experimental aspects of the SIM project will begin next year.

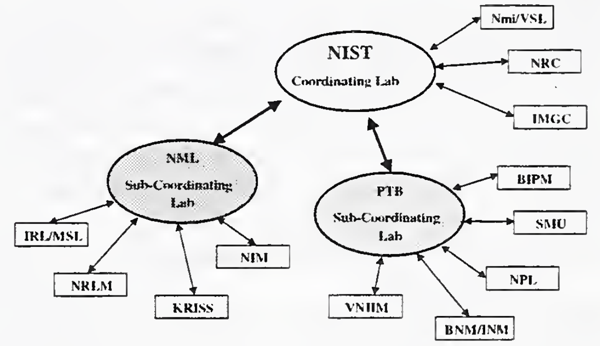


Figure 1. CCT Key Comparison 3: 83.8058 K to 933.473 K

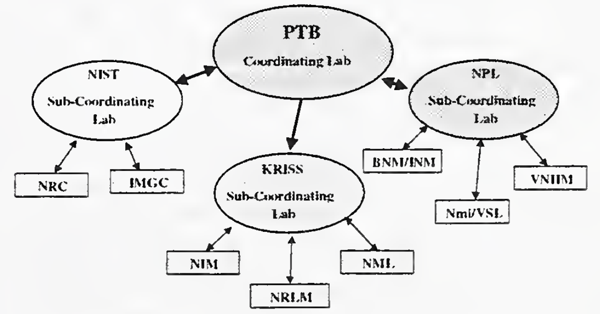


Figure 2. CCT Key Comparison 4: 933.473 K to 1234.93.K

5. *Standards for Low Concentrations of Water Vapor in Gases*

***J. T. Hodges**, P. H. Huang, G. E. Scace, B. W. Mangum, and D.C. Hovde (Southwest Sciences)*

Objective: To provide absolute standards for water vapor generation and measurement in the concentration range 1 to 1000 nmol H_2O/(mol of carrier gas).

Problem: Strict monitoring and control of trace quantities of water vapor are required in numerous industrial processes related to the fabrication of microelectronics, photonics and semiconductors. Regrettably, metrology-grade standards are not well established in this range, and existing hygrometers are inadequate as they often suffer from hysteresis, irreproducibility and relatively slow response. Further, measurements of low levels of water vapor are complicated by poorly understood interactions between the water vapor, carrier gases and transfer lines, as well as uncertainty in thermodynamic properties of water vapor and carrier gas mixtures.

Approach: The strategy of this program is to develop standard sources of humidity and complementary methods of humidity measurement spanning the same range. Both the generation and measurement schemes are optimized for stability, and both are based on processes that can be modeled from first-principles so that the respective uncertainties can be estimated with confidence.

Results and Future Plans: A thermodynamically based standard humidity source, known as the Low Frost-Point Generator (LFPG), was developed. Its output is linked primarily to the vapor pressure of ice and therefore is governed by the system temperature and pressure–two quantities that can be precisely controlled and accurately measured. The LFPG delivers water-vapor concentrations as low as 3 nmol/mol, with a long-term stability of better than ±0.2 % in water vapor concentration and an accuracy of better than 2 %. Comparison of the new generator with another long-established NIST standard generator indicates that in the region of overlap of the two systems, the two agree to within the stated uncertainty of the reference generator. The LFPG is now available to provide special test services for clients desiring direct traceability to national humidity standards. Commercial sensors being characterized include chilled-mirror devices, optical absorption spectrometers, electrolytic and capacitive devices, and vibrating crystal transducers. Also, using a stable hygrometer as a nulling device, the LFPG provides a reference to which other precision humidity generators may be compared. This approach enables the measurement of water vapor concentrations differing by less than 1 nmol/mol. At present, a comparison of several standard generators (based on permeation tube/flow dilution schemes) is underway, spanning the concentration range 10 to 100 nmol/mol.

Two techniques based upon absorption spectroscopy are being developed for absolute measurement of water vapor concentration. With the first approach, called wavelength modulation spectroscopy (WMS), we demonstrated relatively fast time response, linearity over two decades of water-vapor concentration in the range 3 to 3000 nmol/mol, and precision of better than 1 nmol/mol. The other laser absorption technique being developed is cavity-ring-down spectroscopy (CRDS). It is expected to be more accurate than WMS, as it provides a fundamental measure of water vapor concentration linked to measurements of time and frequency and referenced to the triple point of water.

6. *Comparison of International Pressure Standards*

***J.P. Looney**, A.P. Miiller, J.W. Schmidt, C.R., Tilford, and A. Lee*

Objective: Establish functional agreement between national pressure and vacuum standards for pressures between 3×10^{-7} Pa and 500 MPa.

Problem: International trade is, in part, based upon the equivalence of measurements. Some of the largest segments of industrial measurements are in the areas of pressure and vacuum. To help alleviate technical trade barriers, the relative agreement of national pressure and vacuum measurement standards needs to be assessed, established, formally recognized, and maintained.

Approach: We address these issues through participation in international comparisons of measurement capability with select National Measurement Institutes (NMIs) and regional metrology organizations. In 1996, the Consultative

Committee for Mass and Related Quantities (CCM) identified six Key international comparisons in Pressure and Vacuum measurements, their ranges, the transfer standards to be used, and the NMI that would act as the pilot laboratory. At the 7th CCM meeting (May 12-14, 1999), no new Key comparisons were approved, although a differential comparison was formally adopted as part of Key comparison number 4.

Regionally, we are also the pilot laboratory for a pressure comparison for SIM, and are participants in another.

Results and Future Plans: Key Comparison 1 is organized under the Low and Medium Pressure Working Group of the CCM. The transfer standards package that was developed at NIST, consists of two spinning rotor gauges, two glass Bayart-Alpert gauges and a Stable-Ion gauge, and began circulation among the participants early this year. The comparison is on schedule for a FY 2000 completion.

Key Comparisons 2 and 3 are organized under the Low and Medium Pressure Working Group of the CCM. The transfer package that was developed at NIST. consists of four differential pressure transducers (two capacitance diaphragm gauges and two resonant silicon gauges) and an integral ion vacuum pump to establish the necessary reference vacuum for absolute pressure comparisons, and is also used for the differential comparison. All participants have completed measurements, which formally concludes this comparison on schedule. Data analysis, Draft A report preparation, and report circulation among the participants will be conducted in FY 2000.

Key Comparison 4 is organized under the Low and Medium Pressure Working Group of the CCM. The transfer package has begun circulation among the participants. Although this comparison is somewhat delayed, NIST completed its measurements, on time, at the end of FY99.

Key Comparisons 5 and 6 are organized under the High Pressure Working Group of the CCM. All measurements were completed in FY99. The pilot laboratories are analyzing the data, and preparing Draft A reports for circulation.

Within SIM, we piloted an atmospheric pressure comparison among five sub-regions and completed measurements in FY99. In FY 2000, data from this comparison will be analyzed and the findings disseminated. In addition, NIST is a participant in a CENAM-led SIM/NORAMET comparison to 100 MPa, which should begin in FY 2000.

	Pressure Range	Transfer Standard	Pilot Laboratory	Participant NMIs*
1)	$3\mathrm{x}10^{-7}$ Pa to $1\mathrm{x}10^{-3}$ Pa (absolute)	Spinning Rotor and Ion Gauges	1	1, 2, 5, 6, 8, 9
2)	1 Pa to 1 kPa (absolute)	Low pressure transducers	1	1, 2, 7, 5, 10, 8, 9
3)	1 Pa to 1 kPa (differential)	Low pressure transducers	1	1, 10, 2, 5, 11
4a)	10 kPa to 120 kPa (absolute)	Pneumatic piston gauge	2	1,2, 12, 13, 10, 6, 5,14,15, 16, 17, 18
4b)	10 kPa to 120 kPa (differential)	Pneumatic piston gauge	2	1,2, 13, 10, 6, 5,14,15, 16, 17, 18
5)	50 kPa to 7 MPa (gauge)	Pneumatic piston gauge	3	1, 2, 3, 4, 5, 6
6)	50 MPa to 500 MPa (gauge)	Hydraulic piston gauge	4	1, 4, 8, 9

***Legend of NMIs:**

1	National Institute of Standards and Technology (NIST), USA
2	National Physical Laboratory (NPL) – Teddington, United Kingdom
3	Laboratoire National D'Essais (LNE) -France
4	National Research Laboratory for Metrology (NRLM), Japan
5	Instituto di Metrologia "G Colonnetti" (IMGC), Italy
6	Physikalisch-Technische Bundesanstalt-(PTB) Braunschweig, Germany
7	Physikalisch-Technische Bundesanstalt-(PTB) Berlin, Germany
8	Korean Institute of Standards and Science (KRISS), South Korea
9	National Physical Laboratory (NPL), India
10	CSIRO-NML, Australia
11	MSL, New Zealand
12	Bureau International des Poids et Mesures (BIPM)
13	Insitut National de Metrologie (INM).France
14	National Research Council (NRC), Canada
15	NMi, Netherlands
16	OFMET, Switzerland
17	INM, China
18	VNIIMS,Russia

7. *Development of Quantitative Measurements for Vacuum Process Control*

C.R. Tilford and T. Gougousi (Univ. Maryland)

Objective: Support semiconductor process-control development with improved residual gas analyzers (RGAs), and *in-situ* RGA and gas flow calibration techniques.

Problem: The increasing volume and complexity of vacuum processing requires real-time process monitoring and control of process gases, reaction products, and gaseous contaminants. Residual gas analyzers are the most promising candidate for this task, and are already used in a variety of vacuum processes, but their often-unpredictable performance has so far limited these applications. Realizing their potential requires a better understanding of the factors limiting their performance, and the development of *in-situ* calibration techniques for highly reactive process gases.

Approach: Develop an *in-situ* sampling and process-gas calibration technique for RGAs and deploy this in collaboration with the University of Maryland (UMD) to check the viability of using RGAs for process control of a semiconductor fabrication tool (*i.e.*, tungsten deposition process).

Results and Future Plans: The *in-situ* gas sampling system was installed on the UMD tool, which allowed the RGA to directly monitor the tungsten deposition process gases in real-time. The wafers subsequently produced were then analyzed for the mass of tungsten actually deposited. A linear correlation between the amount of deposited tungsten and integrated RGA signals for both H_2 and HF, which are by-products of the deposition process, have been repeatedly demonstrated for multiple batches of ten or more wafers, with each batch processed on different days. For some batches the total deposited tungsten varied from wafer to wafer by as much as an order of magnitude. Results and analyses are still being refined, but to date the best results have been obtained with HF, with variations from a linear curve bounded by about ± 5% (Figure 1). The H_2 data (which are small differences between two large numbers) have about twice the noise (Figure 2). However, during these experiments the RGA sensitivity changed by as much as two orders of magnitude, and reliable results depend on cold-wafer calibration runs between each hot or process wafer. This will be unacceptable for industrial use. This will be addressed with the *in-situ* RGA calibration system, which was recently installed. After a short period of troubleshooting with the calibration system, preliminary experiments indicated that gas phase reactions, and more specifically, ion-molecule reactions occurring in the ion source of the RGA, are responsible for the generation of the relatively high HF background.

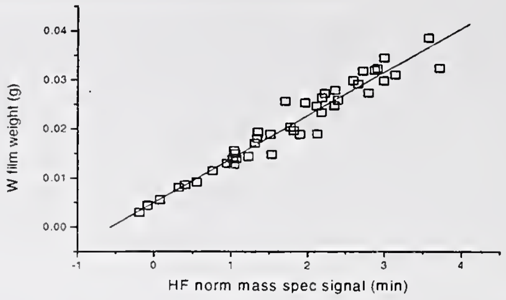


Figure 1. The tungsten film weight vs. overall HF production, which equals the normalized HF concentration multiplied by the tungsten deposition time.

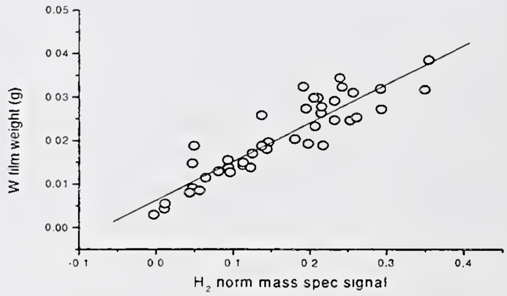


Figure 2. The tungsten film weight vs. overall H_2 depletion, which equals the normalized H_2 concentration multiplied by the tungsten deposition time.

More experiments are planned to provide an independent calibration of the RGA that will allow us to remove the unproductive background runs in between film depositions (we are currently running every other wafer without heating so as to get a baseline measurement of all signals relevant to metrology). Success in exploiting the calibration system in a real process environment is a key challenge to mass spectrometry-based process metrology in rather highly reactive systems such as tungsten CVD.

8. *Improved Gas Flow Standards*

***J. D. Wright**, P. I. Espina, A. N. Johnson,*
G. M. Kline, P. I. Rothfleisch

Objective: Reduce the uncertainty of NIST gas flow standards to 0.05% or less over the range of 1 L/min to 100 L/min. Implement two or more largely independent gas measurement techniques so that internal comparisons can be made between the gas flow standards, validation through improved understanding of systematic, Type B, error sources.

Problem: NIST flow standards in the 1 L/min to 1440 L/min flow range are a set of piston and bell provers first commissioned in the 1960's. In spite of continuous maintenance and improvements, their age and principle of operation hinder reductions in uncertainty below the present levels of about 0.2%. Also, the piston and bell provers require a well-trained operator and do not lend themselves to automation. Meanwhile, other U.S. secondary metrology laboratories and several foreign national metrology laboratories have developed gas flow standards with uncertainties in the 0.04% to 0.08% range. Improvements in NIST gas flow standards are necessary to regain "best in the world" status. U.S. meter manufacturers are starting to produce flowmeters with reproducibility below the 0.2% level, hence for some flowmeter types, the limitation on flowmeter uncertainty is the available flow standards, not the flowmeter performance. Without improvements in the NIST gas flow standards, the competitiveness of U.S. products in foreign markets is at risk.

Approach: The project calls for an iterative approach to design, build, and analyze the uncertainty of a High Accuracy Gas Flow Standard (HAGFS). The new flow standard will be comprised of two volumetric methods and a gravimetric standard combined into one system. In a volumetric system, the flow rate can be determined using Pressure-Volume-Temperature-time (PVTt), and/or Rate-of-Rise (RoR) methods. In a *PVTt* system, the pressure and temperature (before and after the collection interval) are used in conjunction with an equation of state and a carefully measured tank volume to calculate the flow rate. In a *RoR* system, the time derivatives of pressure and temperature in the tank are measured during the gas collection and used to determine the flow rate. In the *gravimetric* version of the flow standard, the mass of gas diverted into the collection tank is measured by placing the tank on a scale. The agreement between the three methods can be used to quantify "B" type uncertainties for each technique.

Results and Future Plans: In the past year, the PVTt and RoR techniques were implemented in a system appropriate for the 1 L/min to 100 L/min range, and two major iterations of the apparatus have been constructed. The first iteration had an uncertainty of 0.3%, while the second iteration reached 0.07% uncertainty. A data acquisition and control program was written which automates the new flow standard. Thanks to the new program, it is now routine to gather test data unattended or overnight in both the new flow standard and the previously existing large PVTt flow standard (800 L/min to 78,000 L/min). The new flow standard and software have been used along with networking software to demonstrate the operation or observation of a flow calibration facility from a remote location via the internet (tele-presence). Demonstrations of tele-presence were made to the National Automated Manufacturing Testbed in 11/98 and to the Director of NIST in 2/99.

A detailed spreadsheet-based uncertainty analysis was developed for a PVTt system. This new uncertainty model has demonstrated what uncertainty goals must be met in the various flow standard subsystems in order to achieve the 0.05% uncertainty goal and it has proven to be a valuable design tool. Tests conducted on sensor subsystems over the past year have demonstrated that the uncertainty goals for pressure, temperature, and volume of 0.01% or better can be met in the final version of the flow standard.

Much has been learned about the diverter valve / timing issue and thermal equilibrium topics which are critical to attaining the 0.05% uncertainty goal. Regarding the diverter valve issue, we have learned that there is a short period of time while the flow is being switched, when flow is dead-ended. During this time, mass accumulates in the inventory volume and flow work significantly heats the gas in the inventory volume. To resolve these fast changes in temperature and pressure and obtain accurate mass determinations for the inventory volume, fast time response temperature and pressure sensors are required. Our previously described uncertainty model differs from previous work in that it is the first analysis which takes into account the issues of flow work and sensor time response.

During the coming year of the project, the final version of the 1 L/min to 100 L/min volumetric system achieving the 0.05% uncertainty goal will be completed. Then the design will be scaled up to cover flows up to 1600 L/min. Computer modeling and further experimental investigation of some thermal and flow phenomena that affect uncertainty will be performed. Also in the coming year, the design of the gravimetric system will be developed, facilitating cost estimates for the next budgetary cycle. With the volumetric standard completed, a thorough evaluation will be conducted, including comparisons with the existing NIST flow standard.

9. *The Effects of Gas Species on Metering Performance of Critical Flow Venturis*

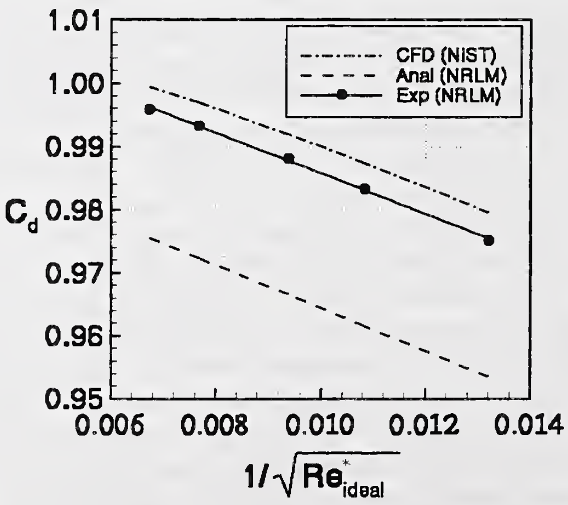
***A. N. Johnson**, J. D. Wright, S. Nakao (NRLM), C. L. Merkle (Univ. Tenn. Space Inst.), and M. R. Moldover (838)*

Objectives: (1) To investigate the potential of using Computational Fluid Dynamics (CFD) to study the flow physics and calibration characteristics of critical flow venturis (CFVs), and (2) to extend the useful range of mass flow and gas compositions for which existing mathematical models can be used reliably for CFV performance predictions.

Problem: Experimental data indicate that calibration curves (plots of discharge coefficient vs. Reynolds number) depend on gas species at low Reynolds numbers. For example, over a Reynolds number range from 2,000 to 40,000 the discharge coefficient of CO_2 differs from that for N_2 by about 3%. Current mathematical models, based on analytic methods, exhibit only a weak dependence on gas species, and therefore have been unable to accurately predict mass flow for all gases. The agreement between these analytic models and experimental data lies within 0.5% for several common gases (*e.g.* N_2, Ar, and air), but has larger errors, exceeding 2%, for other gases (*e.g.* CO_2). The phenomena responsible for this species dependent behavior must be understood and characterized in order to reliably use mathematical models for a wide variety of gas compositions. Furthermore, characterizing these phenomena is an essential step toward developing species independent dimensionless calibration parameters. An immediate benefit of such parameters would be the ability to apply the calibration data of innocuous gases to toxic or corrosive gases, which cannot be measured using standard calibration procedures.

Approach: Computational models were developed to obtain quantitative mass flow predictions over a wide range of Reynolds numbers and gas compositions. Using numerical techniques, we were able to include more of the pertinent physics, thereby eliminating many of the simplifying assumptions commonly used in the existing analytical models. As a first step, the CFD was used to investigate standard hydrodynamic explanations that might be responsible for the species effect. Next, the computational model was extended to include non-standard effects, such as possible vibrational non-equilibrium flow. Molecular theory was used to determine the rate of relaxation of vibrational energy modes due to non-equilibrium processes. The solution of the non-equilibrium flow field was obtained by globally iterating between gas dynamic equations and the vibrational energy rate equation so that both are simultaneously satisfied.

Results and Future Plans: For all but CO_2, the standard CFD model was in good agreement with experimental data (within 0.4% of reading). For CO_2, vibrational relaxation effects significantly affected mass flow. By using the vibrational non-equilibrium CFD model, the error in mass flow predictions for CO_2 was reduced by a factor of five over previous analytic models (see Figure). The vibrational non-equilibrium CFD model was vali-



Comparison of CO_2 calibration curves dated by two independent experiments that varied the vibrational relaxation time of CO_2 and measured mass flow. In the future we plan to study how non-equilibrium phenomena affect other gases to further improve quantitative mass flow predictions of CFVs.

Publication:
Johnson, A. N., Wright, J. D., Nakao, S., Merkle, C. L., Moldover, M. R., ***"The Effect of Vibrational Relaxation on the Discharge Coefficient of Critical Flow Venturis,"*** Flow Meas. and Inst., in press.

10. *Telecalibrations*

***P.I. Espina**, J. D. Wright, P. I. Rothfleisch, G. M. Kline, D. B. Ward, T. T. Yeh, C. Letton (Daniel Industries), S. Oscella (PRC), and W. Seidl (CEESI)*

Objective: Extend the range of NIST gas flow calibration services to meet U.S. industrial traceability to national standards needs. Establish equivalency of national gas flow standards for flow conditions needed by the U.S. gas measurement industry.

Problem: Even though the NIST Fluid Flow Group (FFG) provides calibration services for five decades of flow, U.S. industry has critical needs for gas measurement traceability at much larger flow rates (ranging to $10^5 m^3$/min.) with a wide range of working pressures (1 atm – 60 atm) for many gas species. The construction of facilities capable of handling such large flow rates at NIST is impractical due to capital and space constraints. However, a small number of secondary metrology laboratories in the U.S. are capable of calibrating gas flow meters at flow rates much larger than those attainable by NIST, although with no direct flow traceability to a national standard.

Approach: Current internet capabilities and technological advances in ultrasonics and in pattern recognition now offer the potential for NIST gas flow metrologists to extend the range of capabilities by working with other, larger U.S. gas flow laboratories, *e.g.*, the Colorado Engineering Experiment Station, Inc. (CEESI). These technologies provide the means to take sufficient metrological control of calibrations performed at sites remote to NIST and to certify results as though performed with facilities at NIST. Detailed assessments of all of the pertinent factors that influence meter calibrations are performed. The complete system used determines the volumetric flow rate through the meter being calibrated must be satisfactorily controlled. It also means that the flow profile entering meters to be calibrated is monitored in real-time and approved as being adequate to requirements. To achieve these goals, we have entered into CRADAs with CEESI, Daniel Industries, and PRC. The program with CEESI will produce the required control and certifications of their volumetric flow determination system. The program with Daniel Industries will produce the ultrasonic flow diagnostic unit to monitor pipe flow profiles, and the program with PRC will produce the required, high speed, flow profile assessment capability. Our strategy is to design and implement the complete system on our own PVTt gas flow measurement standard, and to then transplant it to CEESI.

This capability could be used to extend NIST's capabilities to other fluid and flow conditions. It could also be used to establish equivalencies of the flow standards among the NMIs.

Results and Future Plans: We have initiated three CRADAs. The CRADA effort with CEESI is designed to begin with our demonstration of remote internet control on our PVTt gas flow facility which operates like the one at CEESI. The CRADA effort with Daniel Industries has produced the design of an ultrasonic flow diagnostics unit with which we can assess our strategies for monitoring and assessing pipe flow profiles. The CRADA with PRC has initiated the efforts to use multiple travel-time ultrasonic transmissions through non-ideal pipe flows to recognize distortions that have been determined in NIST's previous programs on Flow Meter Installation Effects.

Early in FY99, a National Instruments™ data acquisition system replaced the aging control system used to operate our Pressure-Volume-Temperature-time (PVTt) gas flow standard. New pressure, temperature, and time instrumentation were installed to improve the accuracy of the calibration facility. Using LabVIEW™, a complex data-acquisition/control/analysis program was written to operate the PVTt facility. The program is capable of receiving instructions from a calibration-schedule-file and later performs the calibration in the absence of an operator. To enable the remote monitoring/operation of the facility, MS NetMeeting™ V.2.1 was used to broadcast the user interface of the

PVTt system to other computers at remote locations. Using this system, we provided numerous demonstrations by operating the flow standard from locations within, and outside, NIST.

A distributed control design was suggested that makes use of eight IEEE 1451 network-capable application processors (NCAPs), all connecting to the control computer over a TCP/IP network. Each NCAP will communicate with up to 255 sensors and/or actuators in the PVTt system providing them "smart" functionality. The first Hewlett-Packard NCAPs are being used to control a 14-channel digital flow control device similar to the one projected for the PVTt facility.

In the next twelve months, the project will focus on the implementation of the telecalibration technology at the remote site in Colorado. Issues dealing with the use of Internet-based collaboration tools over secured networks will be of paramount importance. So far, the NIST effort has been so compelling that other NMIs (*e.g.*, PTB – Germany and NMi – The Netherlands) and the U.S. Air Force have seen fit to commence similar telecalibration programs to enable the expansion of their flow traceability services.

Publications:

Espina, P. I., ***"Tele-Metrology: Flow Metrology and the Internet Join Forces,"*** Flow Control Magazine, V, Jan 1999.

Espina, P. I., ***"Tele-Calibration of Gas Flow Meters,"*** Proceedings of the 1998 Measurement Science Conference (MSC: Anaheim, CA).

Espina, P. I., ***"Flow Tele-Metrology,"*** Proceedings of the 1999 IMTC (IEEE: Venice, Italy).

Espina, P. I., Rothfleisch P. I., Yeh, T. T., and Osella, S. A., ***"Tele-Metrology and Advanced Ultrasonic Flow Metering,"*** Proceedings of the 4th International Symposium on Fluid Flow Measurement, Denver, June 27-30, 1999.

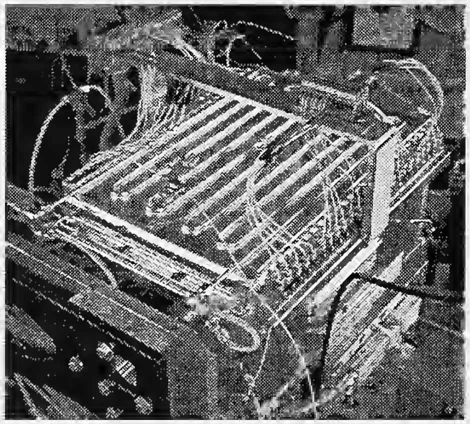
11. *Surface Temperature Measurements*

***K.G. Kreider**, C.W. Meyer, B.W. Mangum,*
W.C. Ausherman, D.P. DeWitt (844) and
B.K. Tsai (844)

Objective: To improve the accuracy of surface temperature measurements, with emphasis in the area of rapid thermal processing (RTP) of semiconductors.

Problem: The semiconductor manufacturing industry requires improved accuracy in measuring the temperature of silicon wafers during processing because accurate temperature measurements are critical to product quality and device performance. As a result, the industry has a requirement of an uncertainty of ≤ 2 °C at 1000 °C for RTP for the next generation of wafer processing.

Approach: Radiation thermometers are used in RTP. Using Si wafers instrumented with combinations of stable thin-film and Pt/Pd wire thermocouples (TCs), we will calibrate light-pipe radiation thermometers (LPRTs). The thin-film TCs minimize errors from heat transfer that would be present for other types of temperature sensors. This technique permits an uncertainty of less that 1 °C when the wafer temperature is uniform to within 10 °C. The effect of the temperature of an LPRT itself on the temperature it measures will be investigated to determine the proper procedure for calibrating an LPRT.



RTP Tool, external view of lamp housing (dimensions of 38 cm x 50 cm)

Results and Future Plans: The stability and performance of thin-film TC material systems (Pt, Pd, Ir, and Rh) were evaluated during FY99 in our thin-film calibration apparatus and during more than 50 heating cycles in the NIST RTP tool. We have correlated their performance, morphological changes observed with optical and electron microscopy, and diffusion profiles measured by secondary ion mass spectrometry depth profiling and reported the results at an international conference, RTP '99. Thin-films of Ir, Rh and Ru on Si coupons have been studied only in calibration tests; they performed well up to 980 °C. Calibration wafers fabricated with Rh/Ir and Ru/Ir thin-film TCs will be evaluated in the NIST RTP tool in FY 2000. In FY99, the expanded uncertainty of the calibration of thin-film TCs was reduced to 1 °C by the technique of welding wire TC measuring junctions to the thin-film coupons used for calibration of the thin-film TCs.

Using cold reflective shields of different configurations to surround the enclosure beneath the wafer to block stray radiation and increase the *effective* emissivity of the TC-instrumented calibration wafer, LPRT calibrations were performed and the results were used for validating models describing the radiation environment of the chamber. The NIST TC-instrumented calibration wafer was in the NIST RTP tool for calibrating radiometers up to 900 °C. Using this technique, we reduced the combined uncertainty to about 2 °C. During FY 2000 we will extend the measurements in the RTP tool to 1000 °C and evaluate the effect of various wafer emissivities on the calibration.

In FY 2000, we also will study the effect of the temperature of an LPRT on the temperatures it indicates. One experiment will involve heating a light pipe in a furnace to 900 °C while it measures the temperature of a constant light source. In the second experiment, a water-cooled sleeve will be placed around the light pipe and thereby prevent the heating of the light pipe as it is inserted into a blackbody source for calibration.

As part of a CRADA with SEMATECH and as a step in transferring our technology to the commercial sector, we have designed, fabricated, tested and delivered two TC-instrumented calibration wafers to SEMATECH for their evaluation in their RTP tool.

12. *Measurements and Models for Plasma Processing of Semiconductors*

M. Sobolewski, K. Steffens, D. Green, J. Olthoff (811), Y. Wang (811), L. Christophorou (811), E. Benck (842)

Objective: Develop advanced chemical and electrical measurement methods and models needed to characterize plasma etching and deposition processes important to the semiconductor industry, enabling continued progress in process optimization, process control, and model-based reactor design.

Problem: Plasma processing reactors have historically been designed and operated using empirical methods alone, but continued evolution of these tools requires a much greater reliance on process and reactor modeling. Indeed, model-based process design and control is an important need identified in the *National Technology Roadmap for Semiconductors*. To obtain more reliable predictions of the spatial uniformity, chemistry, and electrical properties of processing plasmas, further progress in model development and validation is required. Also, to enable improvements in process control, a need exists to develop sensors that are compatible with the manufacturing environment.

Approach: Our experimental program has made use of reference reactors as a testbed for validating models and testing new measurement techniques. The reactors, known as Gaseous Electronics Conference Radio-Frequency Reference Cells (GEC Reference Cells), provide a well-defined basis for comparison of measurements between laboratories. The cells are equipped with a wide variety of plasma diagnostic tools which measure the chemical, physical, and electrical properties of the plasma. Information provided by the set of diagnostics allows testing of models. Also, sensors designed for manufacturing environments can be installed on the cells and compared with diagnostic results.

Results and Future Plans: Work continues on the development of sensors for real-time monitoring of ion current and ion energy in plasma reactors. At present, the development of rf-based ion current and ion energy sensors is limited by a lack of validated models for the electrical properties of plasma sheaths. This year, a sheath model derived last year was tested by experiments and found to be accurate. Algorithms for monitoring the ion current using the new sheath model were derived and were found to be an order of magnitude more accurate than previous methods. Work on ion energy sensors is planned for 2000.

We continue to investigate 2-D species densities in the GEC Reference Cell using 2-D planar laser-induced fluorescence (PLIF) imaging. In these studies, the concentration of CF_2 in fluorocarbon etching and chamber-cleaning plasmas is monitored as a marker of uniformity and chemistry for validation of 2-D plasma models. Previous results indicated that plasma spatial characteristics were correlated to the path taken by rf current flowing through the plasma, which could be varied using a variable impedance load attached to one electrode.

This year, studies were performed to determine the effect of various electrode gaps on plasma electrical and spatial characteristics. With smaller electrode gaps, the CF_2 uniformity and density and electrical parameters become less sensitive to pressure, and the current flowing through the plasma is higher. In 2000, we plan to extend our PLIF work to study etching plasmas in a high-density, inductively-coupled GEC cell. Experiments are also planned in a new industrial-scale, inductively-coupled research reactor, large enough to accommodate 300 mm wafers. This year, the aluminum vacuum chamber of the reactor was constructed and anodized. Optical quality quartz windows were designed, obtained, and machined. Layout of the plasma source and gas delivery system will occur in FY 2000.

13. *Models and Data for Semiconductor Processing*

R. W. Davis, D. R.Burgess, Jr. (838), J. Maslar, E. F. Moore, R. Axelbaum (Washington Univ.), and S. Ehrman (Univ. of Maryland)

Objective: Provide the necessary information and scientific infrastructure to enable the application of semiconductor process models and controllers that are well grounded in fundamental chemical and physical laws.

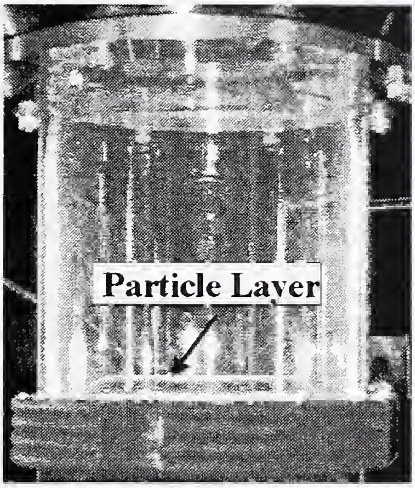
Problem: Reactor and process design are often limited to empirical trial and error approaches that tend to converge slowly, if at all, to semi-optimized states. This implies that important industrial processes are not adequately investigated prior to final implementation. This situation manifests itself in more expensive, lower quality products produced by processes that may be less environmentally acceptable.

Approach: Process simulation has the potential to significantly enhance the design phase of process development so as to improve both efficiency and quality. This is because computational power has evolved to the point where highly sophisticated models can be constructed for a variety of complex semiconductor processes. However, the increasing complexity of these models implies a greater need for accurate fundamental thermochemical and kinetic data, which are not presently available. Our approach is both to develop and use methods for reliably generating the data necessary for process modeling. The reliability, quality, and utility of the generated data must also be demonstrated to the user community. Consequently, the development of process models of wide applicability is essential, as is model validation carried out in reference reactors prototypical of industrial processing equipment. This typically requires the development of reacting flow computer simulations that employ the aforementioned data for input.

Results and Future Plans: Our effort in the area of microcontamination in CVD reactors continued with the further development of the existing moment transport aerosol model for contaminant formation and transport in a rotating disk reactor. Several significant errors were removed from the code, and the range of parameters for which it can provide a solution was noticeably extended. Additionally, a suite of model problems useful for assessing the accuracy of aerosol dynamics algorithms for stagnation-flow reactors was developed. The development of a second, more fundamental aerosol model for this reactor configuration continued at Washington University. A preliminary sectional aerosol model was developed and will be completed during FY 2000.

The experimental effort involving a rotating disk CVD reactor apparatus continued with modifications to improve its vibrational stability and operational safety. Flow visualization was performed over an extended process parameter range in order to determine an acceptable operating regime with uniform stagnation flow. It was found that helium at a pressure of approximately 150 Torr is necessary to meet this criterion. Finally, preliminary experiments were initiated with silane injection. These experiments revealed a highly visible narrow layer of particles in the thermal boundary layer just above the disk. The investigation of this particle layer will be a primary activity during FY 2000.

Our work continued in database compilation for chemical species of importance in semiconductor processing. *Ab initio* and RRKM/Master Equation calculations were carried out of reaction systems involving fluorinated hydrocarbons (etching gases). The theoretical predictions were found to compare favorably with experimental values in the literature.



Particle layer above heated rotating disk

Publications:

Davis, R. W. and Moore, E. F., ***"Two Model Problems for Testing Aerosol Dynamics Algorithms for Stagnation-Flow Reactors,"*** Aerosol Sci. Tech., in press.

Louis, F., Burgess, D. R., Jr., Rayez, M.-T., and Sawerysn, J.-P., ***"Kinetic Study of the Reactions of CF_3O_2 Radicals with Cl and NO,"*** Phys. Chem. Chem. Phys., in press.

14. *Gas Sensing with Microhotplate Sensor Arrays*

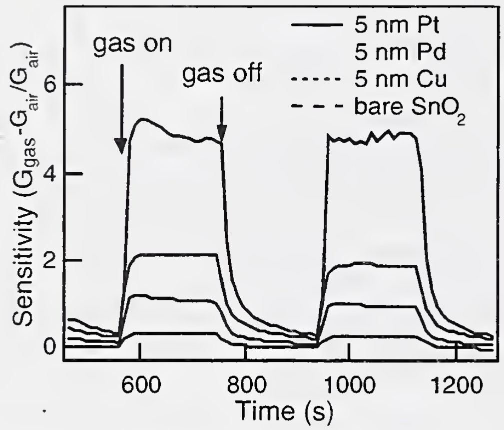
R. Cavicchi, *S. Semancik, R. Walton, J. Allen, M. Aquino-Class, M. Carrier, J. Melvin, and J. Suehle (812); and T. McAvoy, J. Ding, D. DeVoe, and B. Panchapakesan (Univ. of Maryland)*

Objective: Demonstrate feasibility of silicon micromachined, thin-film sensor array technology for multi-analyte, real-time detection and concentration measurement of gases.

Problem: Increasing global competition has placed new demands on the chemical process industry for more efficient use of materials, better process reproducibility, and environmental safety. Meeting these demands requires a low-cost technology for the measurement of gas species, which can provide immediate, on-site analysis for the detection of reaction products, exhaust gases, leaks, etc. Gas-phase measurements are also needed for applications ranging from environmental monitoring at hazardous waste sites to chemical agent detection.

Approach: Advances in microfabrication technology now make possible miniaturization of conventional conductometric low-cost metal oxide sensors into a planar array form. A sensor array platform has been developed which uses a microhotplate as the generic device structure. The microhotplate has three functional layers: a heater, a thermometer/heat distribution plate, and electrical contacts for monitoring the conductivity of sensing films. NIST holds three patents on this technology. There are three key components to the microsensor research program: advancing sensor materials, understanding transducing mechanisms, and developing new methods for sensor operation and signal analysis. Catalyst-doped metal oxide materials are used for sensing films and evaluated on the basis of sensitivity, selectivity, and stability. The dominant sensing mechanisms under investigation include catalyzed reactions, adsorption/desorption, grain boundary diffusion, and electronic effects related to surface states created by chemisorbed species. Surface analytical techniques combined with electrical measurements are used to address these issues. New sensing modes are possible that use the ability of these devices to be heated and cooled in milliseconds over a large operating temperature range (>500 °C). In temperature programmed sensing, the sensor is subjected to a repeating pattern of temperature pulses. Effects which produce a response signal are based on thermally-activated processes, such as adsorption, reaction, and desorption. The sensor generates repetitive response signatures that are characteristic of adsorbed species/sensing material combinations. Neural network and chemometric-based approaches to this problem are being used to optimize the generation of patterns and to analyze signals during sensing.

Results and Future Plans: A study comparing catalysts composed of Pt, Pd, and Cu of different thicknesses showed large enhancements to the sensitivity. Films with 5 nm Pt produced the greatest sensitivity to 50 µg/g methanol in air. A study of sensor materials is in progress. This work will utilize newly fabricated large arrays with 48 sensing elements for combinatorial experiments on sensing materials and large-area structures for studies of sensing mechanisms using mass spectrometry combined with electrical sensing. The suitability of microhotplate sensors for detecting chemical agents is being evaluated. Sensors demonstrated sensitivity to an agent simulant at a level of 200 ng/g.



Conductance response to 50 µg/g methanol in air from four sensors with a SnO2 film with different catalytic layers.

15. *Self-Assembled Monolayers for Diagnostics and Sensing*

***M. J. Tarlov**, G. E. Poirier, and G. B. Saupe*

Objective: To develop the scientific underpinning and measurement methods for using self-assembled monolayers in a variety of chemical and biochemical sensing and diagnostic applications.

Problem: The critical component of chemical sensing and diagnostic devices is the molecular recognition element that imparts both selectivity and sensitivity to the sensor. Self-assembled monolayers (SAMs) are promising chemically selective ultrathin films for sensing and diagnostic applications because of their extraordinary surface chemical tunability. In order for SAMs to reach their full potential in sensing applications, a greater understanding must be established of the factors influencing the self-assembly process and the stability of SAMs, and how molecular scale structure impacts sensing behavior.

Approach: Our research is focussed on SAMs formed by the chemisorption of alkanethiol molecules on gold and other noble metal surfaces. Alkanethiol SAMs are a model system for chemical sensing studies because of their well-ordered and reproducible nature, their tremendous synthetic flexibility that offers precise control of surface properties, and their relative ease of characterization using a variety of surface analytical techniques. Parallel efforts are underway to examine alkanethiol SAMs used in commercially relevant DNA chip applications and to establish a detailed molecular-level understanding of SAM structure and function using scanning tunneling microscopy.

Results and Future Plans: Using a NIST patented protocol, single-stranded DNA is immobilized on gold surfaces through a hexanethiol anchoring group. By addition of a second alkanethiol molecule, mercaptohexanol, DNA monolayers with nearly 100% hybridization efficiencies are achieved. This past year, the effect of DNA sequence length on DNA surface packing was examined using radiolabeling methods. Results indicate two structural regimes for immobilized DNA. For shorter sequences, molecules behave like rigid rods, while longer sequences are better approximated as random coils. These results indicate that quantitatively different hybridization behavior will occur depending on DNA sequence length. Future studies will examine *in situ* the effect of DNA surface structure on the kinetics of hybridization using surface plasmon resonance.

Another critical issue in the application of SAMs is their stability in the laboratory environment. Recent studies have shown, however, that ozone in the air can degrade the SAM film by oxidizing the thiol headgroup. To follow the time evolution of the reaction, SAMs were exposed to increasing doses of pure ozone while recording STM data. These molecular-resolution images reveal that ozone attacks the crystalline monolayers preferentially at the network of domain boundaries between the molecules. As the ozone exposure increases, the reaction spreads into the crystalline domains. These results (see the figures in the Division Overview) point to possible strategies to improve the monolayer's stability in air, such as decreasing the density of domain boundaries or decorating the boundaries with molecules that are inert to ozone. Studies this year will examine the feasibility of using SAM phase transitions as a new transduction mechanism for chemical sensing.

16. *Microfluidic Measurement Technology*

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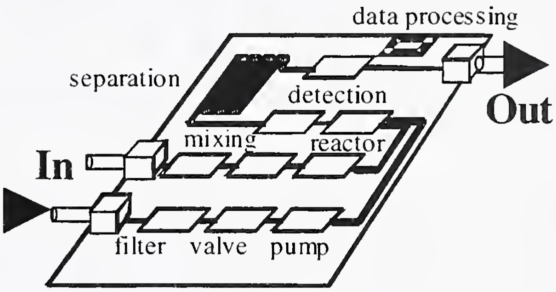
W.A. MacCrehan (839), L.E. Locascio (839),
M.L. Branham (839)

Objective: To address the scientific and measurement issues involving microfluidic dynamic behavior, chemical selectivity, and detection that impede the development of "lab-on-a-chip" devices.

Problem: Microfluidic, or so-called "lab-on-a-chip," devices are generating much excitement because of their potential for high-speed and high-throughput chemical analysis relative to conventional bench-top chemical instruments. Miniaturized devices for DNA separations have recently been commercialized for DNA sequencing. However, expansion of the "lab of the future" to other applications is held back by numerous technical barriers, such as poorly characterized and nonreproducible microfluidic behavior, the high cost of silicon-based devices, and the lack of integrated and chemically selective detection elements.

Approach: In the first year of this multi-year Competence project, methods are being developed to: 1) accurately measure fundamental flow parameters in polymer-based devices, 2) characterize the surface of microchannels, and 3) modify microchannel surfaces for greater reproducibility and tailored applications. Division 831 has developed an inexpensive and simple method for forming microchannels in polymer substrates. Because electro-osmotic flow (EOF) is currently the most widespread method used for moving fluid in microchannels, we are measuring the fundamental EOF parameters of electro-osmotic mobility and flow velocity using a suite of plastic substrates. The EOF is created in the polymer channels by applying an external electric field as in capillary electrophoresis. High surface-to-volume ratio of microchannels cause surface properties to dominate microfluidic behavior and ultimately device sensitivity. To correlate the EOF data with the surface chemical state of the microchannels, surface analysis methods are used to characterize the polymer substrates. Methods are also being investigated for modifying microchannel surfaces including exposure to gas plasmas and deposition of polyelectrolyte multilayers (PEMs). These derivatizations result in devices with reproducible surfaces and allow tailoring of channels for specific chemical analysis problems. For example, PEM coatings on channel walls can prevent the adsorption of proteins or can enable separation of neutral organic compounds that cannot be resolved with conventional electrophoresis.



Lab-on-a-Chip

Results and Future Plans: Microfluidic channels have successfully been made in a number of different plastics. Optically-based and current monitoring methods have been implemented to measure electro-osmotic mobility in different plastic devices. Such fundamental data relating flow to surface properties will enable developers of this technology to tailor plastic microfluid channels for specific applications. Future research will include refinement of the flow measurement techniques, development of derivatized plastic devices, and the use of the derivatized plastic channels for analyte separation.

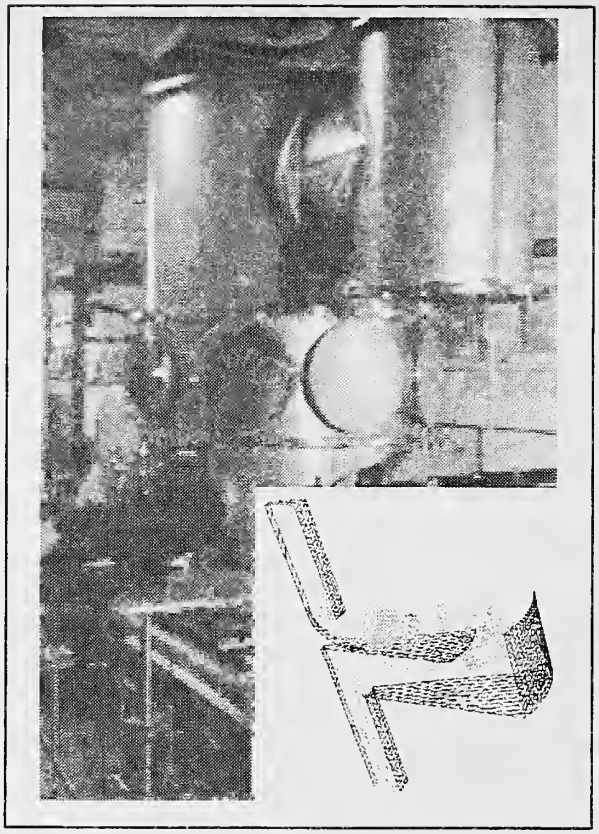
17. *Measurement Technology for Benchmark Spray Combustion Data*

C. ***Presser****, S.R. Charagundla, J. D. Widmann, H. Clack, A.K. Gupta (Univ. Maryland), J. Dressler (Fluid Jets Assoc.), R. Cope (Dow Chemical), J.D. Smith (Univ. of Illinois), M.G. Giridharan (CFD Res. Corp.), S. Menon (Georgia Inst. Tech.), and D. Gera (Fluent, Inc.)*

Objective: Develop measurement technology to provide benchmark experimental data for input/validation of multiphase combustion models, calibration of instruments/sensors, and development of advanced diagnostics.

Problem: Control of process efficiency and the formation of species byproducts from industrial combustion systems (*e.g.*, power generation and treatment of liquid chemical wastes), is relying increasingly on computational fluid dynamics (CFD) simulations to provide relevant process information in a cost-effective manner. However, there is a dearth of reliable data for specifying model initial/boundary conditions, and a need for experimental/numerical comparative analysis of conditions within the reactor. The need to provide benchmark data on the characteristics of the droplet field, flame structure, heat transfer, and particulate/gaseous byproducts, and its interrelationship with the system operating conditions (*e.g.*, desired stoichiometry) is crucial for the development and calibration of advanced computational models, diagnostics, and instrumentation.

Approach: The NIST reference spray combustion facility has evolved into a well-characterized and controlled test bed that can handle different 1) process liquid fuels and wastes, 2) atomizer designs, and 3) combustor configurations. The experimental apparatus consists of a swirl burner enclosed within a stainless steel chamber to achieve improved system operating control resulting in reproducibility of the spray flame characteristics (Cf. Figure). A variety of diagnostics are employed or being developed to characterize the input fuel stream (fuel composition), spray flame (droplet size, velocity, and temperature), and flame emissions (particulates and chemical species). A coupled experimental/computational approach is used to involve modelers from our industrial partners directly in the program.



Results and Future Plans: Our efforts in FY99 focused on dissemination of the benchmark experimental database so that our partners in industry and academia would initiate the computation and validation process, *e.g.*, simulations for velocity (Cf. Figure inset) and gas. A one-day workshop was held with attendees representing industry (chemical, power, energy, and software developers), other government agencies, and academia. The focus of the workshop was to familiarize the participants with NIST's reference spray combustion facility, assess the information currently provided in the database, review preliminary findings from simulations of the NIST facility, allow modelers to express their data needs, and provide an opportunity for feedback concerning future measurements.

Discussions at the workshop focused on the industry needs and what should be the next phase of the program in FY 2000, after completion of the baseline case. A wide range of needs was discussed with some topics prioritized. Follow-up discussions are currently underway with the participants to determine the most critical industrial needs and how we can provide the greatest impact.

18. *Standards for Raman Spectroscopy*

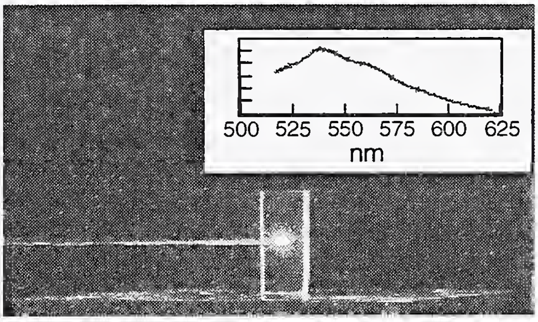
***W.S. Hurst**, J. Maslar, V. Podobedov, S.J. Choquette (839), E.S. Etz (837), D.H. Blackburn (837), and R. McCreery (Ohio State Univ.)*

Objective: Critically evaluates existing approaches and develops new methods and associated standards that will provide for calibration of the frequency and intensity of Raman spectral data.

Problem: It is widely acknowledged that major advances in analytical Raman instrumentation have virtually revolutionized Raman spectroscopic measurement, so that Raman spectroscopy is now finding its place in the industrial environment for process measurements and quality control. Calibration of both the Raman intensity and frequency are important. Intensity calibration is needed to make process-control Raman measurements instrument independent, for analysis of unknown mixtures, and for reliable and robust quantification. Maintaining a highly accurate frequency calibration on all spectra is needed if the training sets of multivariate analyses used in process control algorithms are to maintain their validity. The lack of accepted practices, standards and spectral libraries has been a main obstacle to the acceptance of Raman in industrial settings and is a barrier to its use in the regulated industries.

Approach: One approach for intensity calibration will be to evaluate the use of fluorescent materials. NIST will research the choice of fluorescent materials by evaluating the fluorescence spectra of rare-earth doped glasses to provide both broad-band and narrow-band emissions over the common Raman spectral domains. These glasses will lead to the certification of a set of Standard Reference Materials (SRMs) traceable to NIST primary radiometric standards. While fluorescence can be exploited for intensity calibration, a more fundamental approach rests upon the determination of absolute Raman cross sections to provide an absolute intensity calibration that is verifiably instrument independent. A Raman gain spectrometer will be developed for the measurement of the Raman cross sections of judiciously chosen liquids and solids that may serve as absolute Raman intensity standards. Frequency calibration issues will be studied, with reference to the ASTM adopted Raman shift standards of selected compounds. Contact with the Raman community of major chemical industries, instrument manufacturers, regulatory agencies, and initiatives adopted by the ASTM E13.08 Subcommittee on Raman Standards will be maintained so that methods, standards, and techniques developed by NIST are widely accepted by the industry.

Results and Future Plans: Three stable glass compositions have been identified for each of three laser wavelengths and Raman regimes, 514.5 nm (Ar-ion), 785 nm (Ti:sapphire and diode) and 1064 nm (Nd:YAG, FT-Raman). These glasses provide featureless broadband emissions that appear ideally suited as secondary fluorescence standards (see figure). Importantly, these glasses were used in macro- and micro-Raman measurements, and were found to be homogeneous with no photobleaching upon laser irradiation. Currently, the calibration of the Raman intensity, using radiometric white light sources, is being studied on several spectrometers at NIST. We are also studying on these instruments the consequences of using the fluorescent glasses to transfer the white light calibration. Possible systematic errors in the optical measurement arising from effects such as polarization dependencies, sampling position, collection angle, and spectral resolution, are under study. Presently the calculation of band area ratios from the spectrum of cyclohexane is being used to evaluate the results. A round robin will be conducted in FY 2000 through the ASTM E13.08 subcommittee to ascertain the variability in intensity-corrected Raman spectra that are obtained using fluorescent glasses with relative intensities established at NIST.



Broadband fluorescence from uranyl silicate glass

19. *Quantitative Optical Measurements of Gas Partial-Pressures*

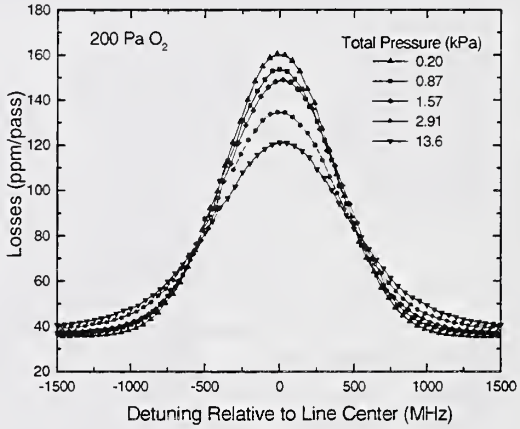
J.P. Looney, J.T. Hodges, and R.D. van Zee

Objective: To develop measurement standards for partial-pressure measurements of gaseous contaminants.

Problem: Because low-level gaseous contaminants often cause deleterious effects in manufacturing processes, a need for sensors with improved sensitivity and accuracy has arisen. For example, the SIA Roadmap identifies the need to measure water vapor at the 100 ppt_v level as key to manufacturing the next generation of devices. Conventional techniques for generating and monitoring low partial-pressures of active gases, such as water, are limited by adsorption and the reactivity of the monitoring devices. Similarly, existing vacuum and humidity standards are incapable of meeting many industrial measurement requirements. The development of a fundamentally new approach to this problem is required.

Approach: Our approach to this problem is to develop quantitative, high-sensitivity optical absorption measurements. Cavity ring-down spectroscopy (CRDS) was identified a promising approach. The centerpiece of CRDS is an optical resonator. The quality of the mirrors that form the resonator determines the time constant with which a pulse of light captured in the resonator decays. Introducing a gas that absorbs the light in the trapped pulse decreases this "ring-down time," and this change is a measure of that species' concentration.

Results and Future Plans: During the past year, which was the final year of NIST Competence funding, our research efforts focused on assessing the precision with which absorption measurements can be made with a unique variant of the CRDS, which we call single-mode CRDS. The performance of the approach proved to be exceptional. Three figures of merit attest to the precision of the single-mode CRDS measurements. The first of these is the relative standard deviation in the mean ring-down time. It was measured to be $\approx 3 \times 10^{-4}$, less than a decade from the limit imposed by photon-statistics. Importantly, the relative standard deviation was the same if calculated from a weighted nonlinear-regression of a single ring-down signal or extracted from an ensemble of hundreds of measurements. This observation indicates drift has virtually been eliminated during the course of a measurement. The second quantity demonstrating the precision of our measurement is the relative standard deviation in the line strength, which was found to be ≈0.1 % during the course of a day's measurements and ≈0.3 % over a period of days (see the figure). Last, the noise-equivalent absorption coefficient was measured to be $5 \times 10^{-10}\ \text{cm}^{-1}\ \text{Hz}^{-1/2}$. This number determines our ultimate sensitivity to all gases and is about three decades better than the number that we first reported. This level of precision should put our detection sensitivity very close to the SIA Roadmap 2001 target for water, a fact that has been confirmed in preliminary experiments.



The pressure-broadened $^{P}Q(9)$ absorption line in the A-band of $^{16}O_2$.

Publications:

van Zee, R. D., Looney, J. P., and Hodges, J. T., "***Measuring Pressure With Cavity Ring-Down Spectroscopy***," Proc. SPIE 3535, 46 (1999).

Looney, J.P., van Zee, R. D., and Hodges, J. T., "***Single-Mode Cavity Ring-Down Spectroscopy for Line Shape Measurements,***" Spectral Line Shapes 10, 275 (1999).

van Zee, R. D., Hodges, J. T., and Looney, J. P., "***Pulsed, Single-Mode Cavity Ring-Down Spectroscopy,***" Appl. Opt., 38, 3951-3960 (1999).

IV. Surface & Microanalysis Science Division (837)

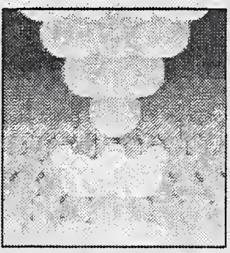
Richard R. Cavanagh, Chief

A. Division Overview

Mission:

As part of the Chemical Science and Technology Laboratory at NIST, the Surface and Microanalysis Science Division promotes U.S. economic growth, safety, health, and environmental quality by working with industry, other government agencies, and standards organizations to develop and apply key technologies, measurements, and standards for spatially and temporally resolved chemical characterization.

The Division is organized into four groups, the Atmospheric Chemistry Group, the Microanalysis Research Group, the Analytical Microscopy Group, and the Surface Dynamical Processes Group, which conduct measurements and research in seven program areas. The Division also benefits from the presence of three NIST fellows who, although not associated with specific groups, interact extensively with a broad cross section of the Division and with organizations internal and external to NIST.



Surface & Microanalysis Science

R. Cavanagh, Chief

[illegible]

This year the Surface and Microanalysis Science Division hosted the first meeting of ISO TC 202 in the U.S. on Sept. 28th - 30th at NIST. ISO TC 202 develops international standards for electron probe analysis, analytical electron microscopy, scanning electron microscopy, and solid-state x-ray detectors. Division scientists currently serve on the TC 202 Technical Management Board and chair two of the three subcommittees. In addition Division scientists are actively involved in the development of standards for inhomogeneity testing of standard samples for electron probe analysis and x-ray data reporting

Division staff also attended the First Environmental Scanning Electron Microscopy (ESEM) Roadmap Workshop and were joined by representatives from Australia, USA, UK, and Canada. The purpose of the Workshop was to determine the present state of experimental and theoretical knowledge of ESEM, especially of primary and secondary radiation behavior, and to define a program of critical measurements necessary to advance the field. The workshop led to tasking of specific participants to assemble basic data sets from critically-reviewed experiments, with the objective of preparing a comprehensive ESEM text emphasizing basic theory, operations and applications that will begin to establish consensus standards in this field.

In a new project "Characterization of Ultra Thin Films," the Division has focused on issues of relevance to the semiconductor industry. Through interactions with EEEL, we obtained samples from SEMATECH (oxynitrides) that have been the subject of an industry-wide round robin. These samples are particularly appropriate, as the industry round robin has generated a rich database on these materials. To date we have measured these by grazing incidence x-ray photoelectron spectroscopy (GIXPS) and multiple voltage electron probe microanalysis (MVEPMA). The GIXPS measurements are particularly appropriate, as they represent a measurement approach that is widely available but poorly quantified in the area of thin film characterization. In addition GIXPS and MVEPMA have been done on thin films of SiO_2 on Si. We are awaiting the delivery of the scanning Auger microscope (expected in March 2000) to complete these measurements. The AEM work on this project has concentrated on developing state-of-the-art AEM sample preparation methods that do not alter the thin-film structure so that accurate thickness measurements can be made. TOF-SIMS depth profiling with a low energy ion beam is also being applied to these samples for comparison. Following the initial work on oxynitrides, we plan to expand the scope of this effort to include oxides and shallow implants (such as boron). Recent discussions with EEEL have brought Ta_2O_5 film characterization into the scope of this project.

Division staff developed a new method for isotopic black carbon analysis. This significant new approach relies on physical separation by selective oxidation or volatilization of the sample. By use of this separation method, a definitive analysis of environmental black carbon appears to be within reach.

Staff attended a NARSTO/EPA meeting and workshop to determine national needs, and solicited feedback from the industrial, OA, and international representatives regarding the NIST role in SRM and measurement development.

Through the engineering of higher throughput probes, Division staff have achieved a factor of five improvement over previous acquisition rates of Raman-NSOM spectra. This improvement has allowed Raman-NSOM spectra and *hyperspectral* images to be recorded with an optical resolution below 100 nm. The Division has advanced the ability to acquire Raman- and IR-NSOM images in an artifact-free manner such that these techniques provide an alternative to transmission electron microscopy probes of complex surface structures which require a chemically specific stain to allow differentiation between the organic species present.

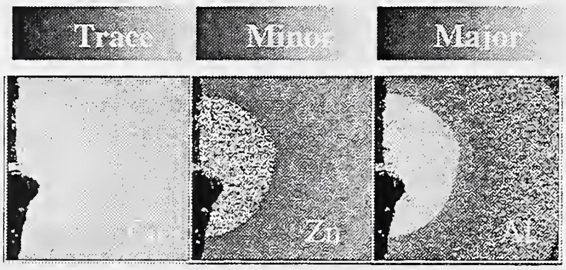
Division Programs

Nanoscale Chemical Characterization

The need for improved spatial resolution currently limits the ability of Industry to answer key questions regarding the chemical composition of surfaces and interfaces. Needs range from improved chemical and structural diagnostics to phase identification and trace compositional analysis. In addition to meeting current industry needs in these areas, there is a continuing demand for new measurement methods to be developed that will be positioned to meet emerging measurement challenges. The Division develops measurement tools that enable the chemical characterization (elements, isotopes, and molecules) at millimeter to nanometer spatial scales with major, minor, and trace concentrations. We strive to develop measurement tools such that the "microspatial" relationships of chemical species can be correlated with specific macroscopic properties. Current efforts have led to:

- Chemical phase identification using electron backscatter diffraction
- Capillary x-ray focusing optics for bench-top x-ray microfluorescence
- Three-band color rendering for quantitative SEM image assessment (Cf. figure below)

For additional information, see Technical Highlights 1-3.



Logarithmic 3 Color-Band ("major-minor-trace") rendering for quantitative SEM image assessment. Diffusion zone formed by penetration of aluminum into brass during failure of a residential electric power outlet (Cf. Technical Report 3).

Particle Characterization and Standards

Particle measurement issues arise in industrial, environmental, and processing environments. Providing appropriate measurement tools for different particle analysis issues is a key focus in the Division. In this program, we direct our efforts to:

- Identify samples, contaminants, or analyze particles in advanced materials
- Apply infrastructure measurement capabilities to characterize actinides and other materials
- Develop standards, data, and measurement protocols for quality assurance
- Transfer measurement capabilities to communities, *e.g.*, DoD, DoE, and IAEA.
- Characterize individual particles
- Automate particle measurement methods, while developing or extending software for data collection, visualization, and interpretation.

Topics of concern this year include:

- Urban Dust standards for EPA filter analysis
- Improved particle characterization in the ESEM
- Characterization of controlled sources of Diesel soot

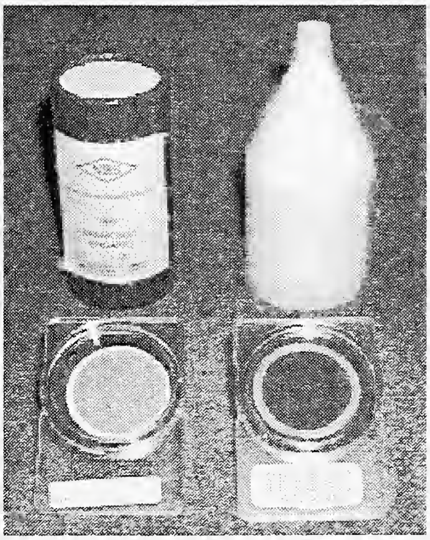
For additional information, see Technical Highlights 4-6.

Electronic and Advanced Materials Characterization

Electronic and advanced materials are increasingly impacted by compositional changes on the submicron length scale. This is routinely seen in the semiconductor area where the ever-shrinking size of device features continues to allow the industry to realize Moore's Law, the exponential increase in transistor density on a chip. Current Division efforts span thin-film characterization, surface chemical growth mechanisms, and novel nanostructures for the electronics industry. Increased interest in optoelectronics can be seen in the activities of this program, with increased efforts being directed toward the development of measurement techniques that address needs which range from those of the Semiconductor Industry Association (SIA) roadmap to the determination of contaminants in starting materials and finished products. Activities this year span silicon-oxynitride dielectric ultrathin films, coatings, and improved electron microprobe procedures for layered specimens and thin films:

- Measurement standards for AlGaAs tech-nologies
- Spectroscopic imaging for magnetic nanostructures
- Effects of high temperature annealing on AlN capped SiC

For additional information, see Technical Highlights 7-9.



Air particulate matter filter Standard Reference Material (SRM 2784) loaded with resuspended Baltimore Dust.(Cf. Technical Report 4)

Measurement Science

Division efforts span numerous challenges in measurement science as they relate to small samples. This may be in the area of small particles, reactions of surface layers of atomic dimension, or minor isotopes that are found in environmental samples. To sustain our position in credible measurements in such vanishingly small samples, the Division invests resources in a range of efforts to provide appropriate quality control and quality assurance capabilities of interest to U.S. industry. Our efforts aid industry in achieving ISO 9000 certification requirements, and provide the basis for traceability among the national and international standards bodies, industries and governments.

Data analysis tools developed in this program advance the application of microanalytical methods. One realization of this has been in the development of the NIST x-ray database and spectral analyzer program DTSA. This year, Division staff used DTSA to simulate the conditions of experiments performed in 1925 by Noddack. Noddack claimed to have discovered element 43, a discovery widely attributed to Perrier and Segre's work of 1937. Simulations were made for a range of compositions under the reported experimental conditions. The model results were compared to the 1925 spectrum and found to support the spectral information of the original work.

Other efforts this year include heterogeneity standards for thin films, Rietveld analysis for the characterization of thin films, and:

- Development of Raman intensity standards (in collaboration with Divisions 836 and 839)
- Standard Test Data for the XPS community
- A nonparametric bootstrapping method for laboratory accreditation
- Nondestructive methods for quantitative thin film characterization
- Standard reference data for XPS and Auger analysis - SRD 20, 64 and 71
- CO_2 reference standards for isotope dilution mass spectrometry

For additional information, see Technical Highlights 10-14.

Surface and Interface Chemistry

Characterization of surfaces and interfaces demands methods that can address the chemical and structural changes that occur in different environments. Surface reactions are central in the area of catalysis, solid-state sensors, adsorption, and electronic device fabrication. The Division provides measurement tools, fundamental data, and models that address the events that occur at surfaces and interfaces during basic chemical reactions. Systems of concern include photon-stimulated reactions at surfaces, the reactions of energetic radicals with surfaces, the growth and structure of thin-films and interfaces, and the formation and structure of buried interfaces. Current activities include:

- Vibrationally-resonant sum frequency generation for the *in situ* evaluation and characterization of interface processes. With this technique, interface reactions under hydrating conditions, as well as structural features of buried interface layers, can be probed.
- Development of tractable theoretical models that provide fundamental insights into electron transfer processes. These processes are known to be critical in a number of surface and interface chemistries, including surface bond cleavage that is instrumental in desorption and catalysis.
- The characterization of electronic effects in oxygen atom reactions with surfaces to probe fundamental aspects of plasma-based surface and interface processes.

For additional information, see Technical Highlights 15-17.

Advanced Isotope Metrology

Isotopic distributions can provide significant information about the sources of nanoscale samples that would be difficult, if not impossible, to obtain using other methods. Examples of the utility of such information ranges include ^{13}C and ^{14}C in aerosol emissions, ^{18}O in drinking water, ^{29}Si in meteorites, and enriched uranium in IAEA swipe samples. Division efforts are concentrated on improving the detection limits for isotopic methods, advancing methods for the discrimination of background signals, and developing protocols to address interfering signals. The Division develops procedures and provides tools to measure isotopic ratios of chemical species in processes such as combustion, in atmospheric gas-particle interactions and transformations, and in contaminant transport.

This year, Division staff collaborated with scientists from Colorado, Nevada, and California in the Northern Front Range Air Quality Study. The main objective of the study was to determine the source of pollution so that Colorado legislators could determine viable strategies to reduce the Denver "Brown Cloud". The ^{14}C analyses conducted by NIST provided a direct, assumption-free capability to discriminate fossil fuel from biomass carbon source apportionment for the study. The results provided policy relevant information for the Colorado legislators and public health officials. Other activities this past year included:

- Improved detection limits for environmental black carbon
- Synergy of SEM and source apportionment in environmental black carbon
- SIMS methods for IAEA sample analysis

For additional information, see Technical Highlights 18-20.

Molecular Scale Chemical Characterization

The need to assess chemical information beyond that of composition and phase is critical for emerging applications in areas including polymer science, catalyst development, and nanoscale device optimization. This program addresses the availability of appropriate measurement tools through efforts to adapt existing instruments and to develop new approaches that meet the projected measurement needs of industry. Improvements in sensitivity coupled with reductions in sample degradation issues are being pursued in Secondary Ion Mass Spectrometry (SIMS) to broaden the impact of this technique to include polymer and other “soft” surfaces. Scanned probe techniques are being assessed for extracting molecular scale information due to their *in situ* capabilities. The ability to develop robust and quantifiable measurement methods and to assess appropriate methodologies are being pursued. Chemical derivatization and phase contrast methodologies in atomic force microscopy are being applied to problems in collaboration with the Biotechnology Division (CSTL) and the Polymers Division (MSEL).

Innovative methods are being developed in our Nanoscale Catalysts Competence Project to combine optical contrast mechanisms with near-field measurements. The Division has developed capabilities in visible, Raman, and infrared near-field methodologies, and has developed collaborative efforts with MSEL, BFRL, PL and other Divisions within CSTL. Efforts this year have led to:

- Robust methods to address topographic artifacts in NSOM

- Initial results from the NIST broad-band infrared NSOM
- SIMS cluster source advances

For additional information, see Technical Highlights 21-23.

Interactions

The Division is involved in advisory and technical efforts with other government organizations including EPA, IAEA, ATF, DoE, and DoD. Close relations are maintained with national (*e.g.*, ASTM, ANSI, MAS, EMSA, NFPA) and international (*e.g.*, ISO, IUPAC, IAEA, VAMAS) organizations. The Division also maintains extensive interactions with private industry including: Ceramem, Charles Evans & Assoc., DOW, Dow Corning, DRI, DuPont, Lucent Technologies, McCrone Assoc., Noran, Peabody Scientific, Photon Imaging, Schafer Vallecitos Laboratories, SRI, 3M, Visteon, and XOS, Inc.

Staff Recognition

- **Robert Fletcher**—1999 SIGMA XI Award for Outstanding service to the NIST chapter, and

- **Dale Newbury**—co-recipient of the 1998 Best Paper award from EEEL for the article "High Resolution Energy Dispersive Microcalorimeter Spectrometer for X-ray Microanalysis," J. Microscopy 188, 196, 1997.

B. Selected Technical Reports

1. *Phase Identification of Individual Crystalline Particles by Electron Backscatter Diffraction (EBSD)*

J.A. Small and J.R. Michael (Sandia National Laboratory)

Objective: To develop a novel approach to the identification of the crystalline phase of individual micrometer and submicrometer particles using the electron microscope and based on the analysis of electron backscatter diffraction patterns.

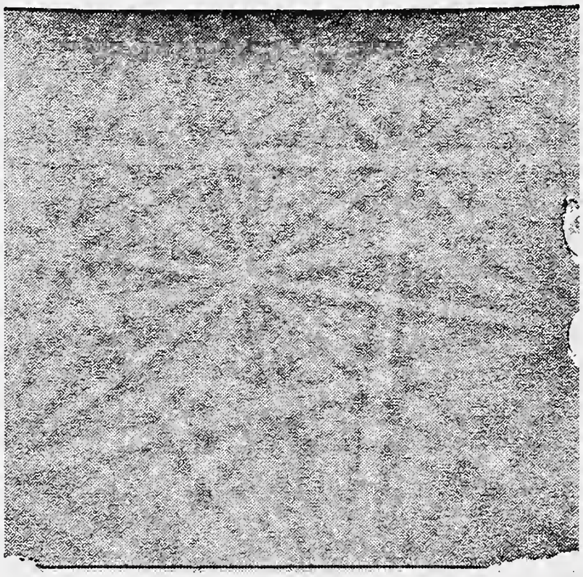
Problem: The effects of particle geometry and size often limit the accuracy and precision of the quantitative electron probe analysis of individual particles by x-ray emission. Typical uncertainties for particle elemental analyses are on the order of ± 10% relative at best which often makes the identification of the chemical phase of the particles difficult if not impossible.

Recently an EBSD Phase ID system has been commercially developed that enables the rapid identification of the crystallographic phase of unknowns in the scanning electron microscope. In this investigation, we looked at the application of the EBSD Phase ID system to the phase identification of individual micrometer and submicrometer particles rather than the conventional EBSD samples that consist of bulk samples with flat polished surfaces. Unlike conventional samples, the phase identification of individual particles may be complicated by several factors related to particle geometry and size. These factors include: difficulty in obtaining flat-field reference images for individual particles, effects of particle mass/size on EBSD image quality, pattern interference from nearby particles or substrates, and the effects of particle composition on EBSD image quality.

Approach: For this study we analyzed a series of particles with known elemental compositions. These particles included U_3O_8 (NBS SRM #950b), PbO_2, SiC, and $PbMoO_4$ (wulfenite). Particles from each of the materials were dispersed onto pyrolitic carbon substrates or carbon tape and analyzed at Sandia National Laboratories in their JEOL 6400 scanning electron microscope at 20 keV acceleration potential. The particles were uncoated and the mounting substrate was at a tilt angle of 70 degrees.

Results and future plans: The results from the EBSD analysis of individual particles indicate that the EBSD system was very successful in identifying the phases of individual submicrometer particles with relatively high average Z as shown in the figure. The backscattered pattern in the figure was taken from a U_3O_8 particle 250 nanometers in diameter that was successfully identified by the Phase ID system as orthorhombic U_3O_8. In addition the results indicate that the analysis of low-Z, submicrometer particles will be challenging because the number of backscattered electrons decreases with decreasing Z. Future efforts in EBSD of individual particles will include studies of particle size and shape effects as well as development of methods to obtain reference images for flat-field processing.

The Phase ID system used in conjunction with an analytical SEM or EPMA provides the analyst with a very powerful and straightforward method to obtain an absolute identification of submicrometer and larger crystalline particles.



Electron backscattered diffraction pattern taken from a 250 nanometer uranium oxide particle. The particle was identified as orthorhombic U_3O_8 JCPDS card # 24-1172.

2. *Capillary X-ray Microfluorescence Instrument*

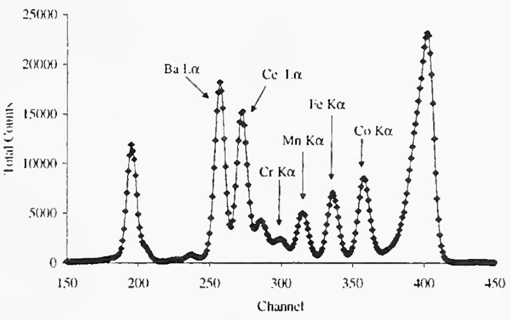
J.R. Swider

Objective: To create, using a laboratory X-ray tube and capillary optics, a flexible, portable X-ray fluorescence instrument capable of sub-nanogram detection with a spatial resolution less than 50 micrometers.

Problem: Although many techniques for microanalysis exist, few are totally nondestructive, easy to maintain, or portable. Although techniques such as Electron Probe Microanalysis (EPMA) deliver sub-micrometer beams, they can be destructive in sample preparation and in analysis, costly, and not portable. Other bulk techniques, such as X-ray Fluorescence (XRF) possess poor beam resolution (millimeter-size) and have detection limits in the µg/g range. Recently the use of synchrotron radiation for XRF has overcome some of these drawbacks in XRF and in charged particle techniques. Synchrotron micro-XRF produces sub-micrometer beams with pg/g detection but still has to be performed at a synchrotron facility. The problem remains how to construct a micro-XRF instrument that utilizes the benefits of X-ray analysis yet is free of the constraints of a large, costly instrument.

Approach: To create a micro-XRF instrument that has low detection limits and is also flexible and portable, the beam must not be wasted with spatial collimation but concentrated with X-ray focusing. Of the many methods used for X-ray focusing, capillary optics best suits a micro-XRF instrument. Capillary optics are based on the premise that X-rays can be reflected at small grazing angles with little loss in intensity. Capillary optic devices are compact, easy to implement, focus a divergent beam well, and conserve the beam brilliance. A polycapillary optic lens ("Kumakhov lens") can be used with a typical laboratory sealed X-ray tube in a relatively small radiation enclosure. The optic is positioned in x,y,z, pitch, and yaw directions to maximize X-ray capture and transmission. The focused beam location and attributes are determined with an X-ray imager. Samples replace the imager at the focal distance and fluorescent radiation is detected with a Si(Li) detector. Motion control, data acquisition, and image acquisition and processing are accomplished on a single PC.

Results and Future Plans: The capillary micro-XRF instrument has successfully analyzed spherical particles around 15 micrometers in diameter as shown below. Detection is in the range of 0.1 ng/g for elements in silicate glasses fluoresced with radiation from a tungsten-anode x-ray tube. The instrument is easily manipulated to accommodate samples in a variety of shapes and sizes and to analyze samples *in situ*. Implementation of a molybdenum-anode x-ray tube will allow more transition and actinide elements to be examined. In order to bring the instrument to a portable stage, the positioners will be replaced with holders fixed to the tube and a small Si-PIN detector will be used for X-ray detection.



15 micrometer Sphere Multicomponent Glass K919

3. *Logarithmic 3-Band Color Encoding: A Robust Method for Visualizing Compositional Information in X-ray Maps Measured in Scanning Electron Microscopes*

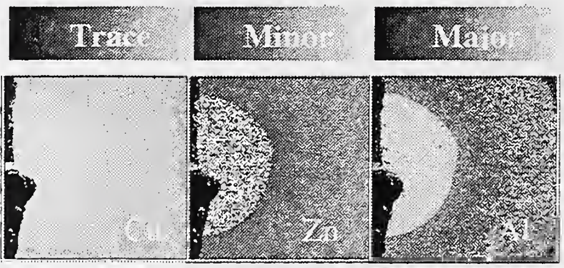
Dale E. Newbury and David S. Bright

Objective: To provide a logical, easily interpretable intensity scale for the presentation of quantitative compositional maps.

Problem: Compositional mapping is one of the most widely used methods of presenting information on heterogeneous microstructures measured with microprobe instrumentation, such as scanning electron microscopy/x-ray spectrometry, analytical electron microscopy/x-ray and electron energy loss spectrometry, Auger electron spectrometry, etc. Existing methods for displaying the compositional axis, especially those incorporated in commercial software systems, are only useful at the most rudimentary level of qualitative analysis with no useful quantitative information available at the pixel level. For example, x-ray mapping systems use a gray level or color value that is related to the raw x-ray count at the pixel location with no corrections for background or relative excitation effects. It is therefore not possible to make sensible comparisons of different elements in the same region or for the same element from different regions or different specimens.

Approach: A new compositional mapping method involves collecting intensity data and making two critical corrections at the individual pixel level. A background correction by simple interpolation from nearby background windows in the spectrum eliminates a critical artifact from measurements of trace (arbitrarily defined as less than 0.01 mass fraction) and minor (0.01 to 0.1 mass fraction) constituents, where the measured "peak" intensity may actually consist primarily of background contributions. Under electron excitation, the x-ray continuum forms a significant fraction of the excited x-ray spectrum, and the continuum intensity is proportional to the overall average atomic number. Thus, when uncorrected x-ray maps of minor and trace constituents are examined, the analyst may perceive apparent compositional contrast which is purely artifactual, arising only from changes in the other constituents and manifested through the continuum dependence on atomic number. The second correction involves the differential excitation as a function of characteristic photon energy. X-ray yield depends strongly on the ratio of beam energy (E_0) to the critical excitation (E_c) energy, $(E_0/E_c)^n$, where n is in the range 1.5–1.7. Because the incident beam energy is fixed, the excitation varies strongly across the photon energy range, typically 0.1 keV to 12 keV. Moreover, the fluorescence yield (the fraction of inner shell ionization events leading to photon emission) depends upon atomic number and the atomic shell. Finally, the x-ray intensity actually measured is modified by the efficiency of the spectrometer, which in the case of energy dispersive x-ray spectrometry decreases strongly for photon energies below 4 keV. All of these effects can be compensated by determining the ratio of the background-corrected pixel intensity to the intensity measured from a pure element (or its equivalent calculated from a compound standard) under identical beam conditions, which yields the "k-value" of classic quantitation procedures. For quantitative display, the resulting k-value maps are encoded with a logarithmic intensity scale using primary colors which grade to pastels, selected such that the blue band covers the range k less than 0.01, green from 0.01 to 0.1, and red greater than 0.1 to 1.0.

Results and Future Plans: The "log 3-band" images have been found to enable the viewer, including non-specialists in the technique, to readily distinguish trace, minor, and major constituents while retaining excellent contrast sensitivity to compositional structures. We plan to extend the technique to incorporate matrix corrections at each pixel to create concentration maps. For other compositional techniques with a wider dynamic range, such as electron energy loss spectrometry in the analytical electron microscope, additional bands will be created to extend the display range to lower trace levels.



Logarithmic 3-Band ("major-minor-trace") display. Diffusion zone formed by penetration of aluminum into brass during failure of a residential electric power outlet.

4. *Resuspension of Urban Dust for Production of a $PM_{2.5}$ Filter Standard Reference Material*

***G. A. Klouda** and H. J. Parish (SRI)*

Objective: To produce an air particulate matter (PM) filter Standard Reference Material (SRM 2784) by resuspending a Baltimore Dust (<2.5 µm aerodynamic diameter) and collecting on quartz-fiber filters for calibration of organic carbon, elemental carbon, and individual compounds of relevance to $PM_{2.5}$ at air monitoring sites throughout the U.S.

Problem: A "prototype" PM on quartz-fiber filters using SRM 1649 Urban Dust was produced in 1995 in collaboration with SRI. SRI's system was designed to resuspend particles in air and collect the aerosol on up to 320 filters simultaneously. For the 300 prototype-PM filters produced, the particle distribution was non-uniform near the filter edge and the PM loading varied up to 20% across filters. These problems were attributed to an inability to reproduce the seal provided by the filter pack thereby causing air to leak across the filter. Also, the inorganic residue remaining from the combustion of the carbonaceous fraction of the "prototype" visually indicated that SRI's system was not breaking up all the larger agglomerates present in the source material. Therefore, to produce a $PM_{2.5}$ filter SRM, it was necessary to develop a custom filter pack to assure that only $PM_{2.5}$ are collected and that the particle distribution is uniform to the filter edge. Also, a contemporary $PM_{2.5}$ sample on the order of several grams would be needed for the production of SRM 2784.

Approach and Results: Through additional funding from SRMP, new filter packs were designed and constructed by URG Corp. to size-segregate particles by impaction and, thereby, allow only <2.5 µm-size particles to reach the filters. Each filter pack included a gasket set and stainless-steel screen to provide an excellent seal and support to obtain a uniform distribution of particles throughout the filter. A test run of SRI's system with the new URG filter packs was completed using a fine coal-mine dust collected on quartz, Teflon® and Nucleapore® (polycarbonate) filters. Visually, the quartz-fiber filters, having a torturous-path, appeared homogeneous throughout. This was verified at the < 3% level through carbon measurements on filter aliquots. However, a visual inspection of the Teflon and Nucleapore membrane-type filters clearly revealed a non-uniform deposit. Tests conducted on a filter pack by URG using a fine-chalk dust showed that the stainless-steel screen had to be repositioned directly behind the filter within the gasket set. A second test run by SRI using SRM 1649a Urban Dust was completed and visual indications were that the quartz and Teflon filters appeared to have a uniform distribution of particles; the Nucleapore filter remains problematic. Carbon analysis of quartz filters will provide the assurance of within-filter homogeneity at an acceptable level and XRF measurements will show how uniform the elemental (inorganic) constituents are across Teflon filters. The variability in the PM loading across-filters remains high, as much as 17%, thus requiring the weighing of each filter before and after resuspension and filtration. For the production of SRM 2784, ~10 g of $PM_{2.5}$ was collected in Baltimore during the fall '98 and spring '99 in collaboration with John Ondov at the University of Maryland.

Future Plans: It is anticipated that the production of 2200 PM filters loaded with Baltimore Dust (SRM 2784) will be completed by the first half of FY 2000. Through an informal release of a modest number of "prototype" PM filters, an immediate need for this material has been recognized and we expect to release it in the fall '99 as a Reference Material. Laboratories that have assisted in the analysis of the "prototype" have also agreed to assist in the certification of SRM 2784. U.S. EPA matching funds are anticipated to help defray some of the cost for production of SRM 2784 as well as support future development of NIST-traceable PM-filter SRMs.

5. *Direct Measurement of Electron Beam Scattering in the Environmental Scanning Electron Microscope Using Phosphor Imaging Plate Technology*

S.A. Wight and C.J. Zeissler

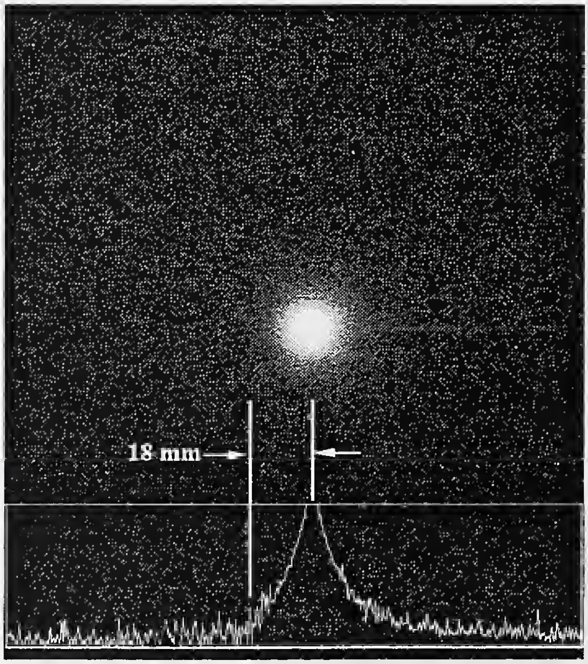
Objective: To measure electron scattering in the environmental scanning electron microscope (ESEM) specimen chamber using phosphor imaging plate technology.

Problem: The scattering of electrons from the primary electron beam, under relatively high-pressure conditions (266 Pa) in the ESEM sample chamber, degrades the analytical accuracy of elemental analysis in the ESEM. The degree of this degradation is poorly known. To date, attempts to experimentally measure the spatial distribution of the scattered electrons have been limited to observing secondary effects such as the intensity of x-rays produced from copper targets positioned at various distances from the primary-electron-beam interaction point. A more accurate distribution of the scattered electron intensity can be obtained from a direct measurement of both the scattered and unscattered electrons over a large area with single electron sensitivity.

Approach: Advances in phosphor imaging plate technology can be exploited for this application. Phosphor imaging plates are sensitive to single electron stimulation, are capable of storing intensity information over several orders of magnitude, and can measure large areas several centimeters in size. The plates have a minimum digitization dimension of 25 µm. The electron scattering area in the ESEM chamber extends over hundreds to thousands of micrometers in diameter. The challenge is to merge phosphor imaging plate and ESEM measurements to accurately determine electron scattering characteristics.

A plate configuration optimized for tritium mapping is ideally suited for mapping of the relatively low energy electrons, 1 to 20 keV, of interest in the ESEM. The plate is placed in the ESEM chamber that is pumped to a pressure of 266 Pa (2 Torr) of water vapor. The phosphor plate is exposed to the 20 keV electron beam for a short time, < 1s, under scattering conditions. After exposure, the plate is retrieved from the ESEM chamber and the intensity distribution of the electron beam and the scattered electrons is recorded.

Results and Future Plans: A phosphor plate image of the primary beam and the scattered electron distribution is shown in below with a linescan through the center overlaid at the bottom of the image. The horizontal line on the right hand side of the image is an artifact of the measurement system. The image shows that the scattered electron intensity is significant, extending approximately 18 mm from the primary beam. Future plans include an electron beam shutter adaptation to reduce the saturation of the primary beam and provide better control of electron dose, and characterization of the phosphor signal as a function of electron energy in the energy range of interest. These measurements will be used to improve theoretical scattering models for the electrons in the ESEM chamber and hence the accuracy of elemental analysis in the ESEM.



Phosphor plate image of primary electron beam and scattered electrons with linescan overlay.

6. *Measurement of Diesel Exhaust by Laser Microprobe Mass Spectrometry*

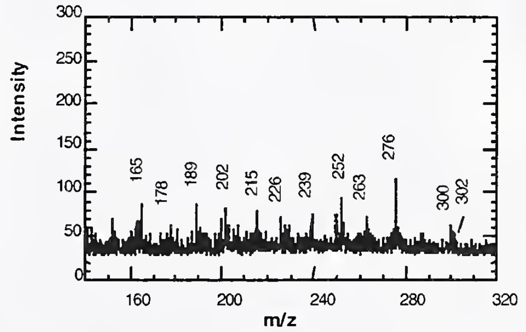
R.A. Fletcher and R.A. Dobbins (Brown Univ.)

Objective: To analyze and compare diesel soot formed from an engine operating at various controlled conditions to that collected from an environmental site and to the NIST SRM material. The ultimate goal is to determine the source of polycyclic aromatic hydrocarbons (PAHs) in engine exhaust products.

Problem: Diesel engines are widely used in trucks, buses and to a lesser extent in automobiles. Diesel soot, a product of the combustion system, is a known environmental pollutant. Diesel soot is a complex mixture of particulate materials primarily composed of black carbon, often referred to as elemental carbon, that is a soiling agent. It also contains many organic compounds including PAHs that are potential carcinogens. Human health and property damage are potential impacts of exposure to diesel particles. To abate soot formation, it is vital to understand the particle formation mechanism during combustion and the source of PAHs, whether from the combustion process or from the fuel. Brown University, NIST and Cummins Engine Company formed a collaboration to study diesel particles emitted from diesel engines operating at controlled conditions.

Approach: Diesel soot particles were collected directly from the output of a test engine operating under controlled laboratory conditions at the Cummins Engine Company. The engine was in all cases warmed to normal operating conditions and operated at normal temperature. Engine operating conditions were closely monitored and the engine parameters that were varied were exhaust gas recirculation and engine speed. The sampling location and methods of analysis required microsampling techniques. The diesel particles were collected on pre-cleaned, uncoated transmission electron microscope (TEM) grids. Special micro-samplers were designed and built at Brown University that could be inserted immediately downstream from the engine exhaust valve. The particles were collected on the TEM grids by thermophoresis. Analysis was accomplished by directly inserting the samples, without further preparation, into a laser microprobe mass spectrometer (LAMMA 500). The material was irradiated with 266 nm radiation to produce ions that were mass analyzed using a time-of-flight mass spectrometer. Approximately thirty mass spectra were taken from each sample. TEM micrographs of the diesel soot were taken at Brown University. The environmental samples were collected from the Fort McHenry tunnel. The tunnel is divided into two tubes -- one for trucks (diesel soot producers) and one for automobiles. The particle samples were collected from the air using a low-pressure impactor that deposited the aerosol on the central region of a quartz coverslip slide that mounted directly in the LAMMA. The SRM 1650 diesel particulate material was analyzed by dispersing a small amount on a quartz coverslip and also on a TEM grid.

Results: We observed significant differences in the mass spectra from three different engine operating conditions. Qualitatively, very little PAH material was observed in samples taken from a warm engine operating with a lean (excess air) mixture ratio. In one engine condition we found spectra indicative of elemental carbon or carbonacous soot. These spectra were very similar to those found for the SRM material. However, certain spectra like the one shown below, taken from a sample of the tunnel air, indicated the presence of PAHs in the m/z range of 165 to 300. These PAH peaks match closely with peaks found in an ethene gas diffusion flame and are common to a wide variety of combustion processes.



Laser microprobe mass spectrum of particles collected from the Fort McHenry tunnel, Baltimore, MD. Peaks corresponding to PAHs are identified. (Sample collected by S. Hoeft)

7. *New Analysis and Correction Procedures that Enable an Order-of-Magnitude Improvement in the Accuracy of Compositional Determinations of $Al_XGa_{1-X}As$ Thin Films*

J.T. Armstrong, R.B. Marinenko, J.G. Pellegrino (812), and K.A. Bertness (815)

Objective: Develop analytical and correction procedures that will enable industrial users to perform routine electron microprobe analyses of the elemental compositions of $Al_XGa_{1-X}As$ thin layers on substrates like GaAs with a relative accuracy of better than 1%.

Problem: $Al_XGa_{1-X}As$ is a semiconductor system with very important properties for a variety of commercially-significant microelectronic and optoelectronic applications. To manufacture working devices that utilize these properties, the composition of the $Al_XGa_{1-X}As$ phase must be known with a precision of better than one percent relative and an accuracy of about one percent relative, *e.g.*, $X = 0.200 \pm 0.002$. Moreover, phases of economic interest encompass well over half of the compositional range in the AlAs-GaAs system. Electron probe microanalysis (EPMA) is one of the most commonly employed analytical procedures for characterization of these materials. However, the accuracy of EPMA in this system is restricted due to the atypically high degrees of characteristic fluorescence and x-ray absorption of some of the x-ray lines used in analysis, as well as the relatively large compositional differences between the analyzed phases and the typically available standards. Different laboratories analyzing the same phases in this system with a variety of commonly-employed commercial analysis programs, obtain compositions that differ from each other and differ from the correct compositions by as much as 20 to 30% relative (instead of the 2-3% relative accuracy often obtainable in EPMA). As a result, many laboratories use empirical corrections or calibration curves relative to internal laboratory reference compounds of uncertified (unknown) composition. Because of this, any inter-laboratory comparison of the relation of electrical or optical properties to composition is subject to serious error.

Approach: We have been working to characterize by EPMA a series of new standard reference materials (SRMs) in the system $Al_XGa_{1-X}As$. Films were synthesized by molecular beam epitaxy with five different known compositions (with 'X' ranging from 0.1 to 0.63) by careful monitoring of the time evolution of the film properties during epitaxial growth. The compositions were determined *by in-situ* optical reflectance spectroscopy and *ex-situ* reflection high-energy electron diffraction (RHEED). The films were then analyzed by high precision EPMA at multiple accelerating potentials. The homogeneity of the films were confirmed and multiple sets of analytical data (including analysis of numerous primary standards) were collected to yield data sets with precisions better than 0.5% relative. We devised a new analysis scheme involving a combination of x-ray lines not typically employed to minimize the uncertainties and artifacts produced by secondary x-ray fluorescence. We determined that there were not enough internally consistent measurements of mass absorption coefficients (MACs) for Al, Ga, and As on which to base the various parameterizations of MACs currently used in commercial correction programs. We added a set of NIST-produced theoretical MACs that appear to be more self-consistent. The data were processed using all of the commonly-employed correction algorithms and the various sets of MACs. We determined the internal self-consistency of the measured ratios of intensities of samples to standards by the a-factor (calibration curve) method and determined a set of superior empirical a-factors for analyses in this system.

Results and Future Plans: The results obtained by processing the same data set with the various combinations of MACs and correction algorithms varied by over 30%. However, when the NIST MACs and the NIST/Caltech "CITZAF" correction algorithms were used, the calculated compositions of all five films agreed with the reflectance/RHEED data within 2% relative (better than 1% relative for four of the five samples). The same degree of agreement is obtained using the best-fit empirical a-factors. This suggests that the level of accuracy required by industry can be obtained using either of these two procedures. Further testing of the accuracy of these procedures is planned as well as a study of III-V semiconductor compounds in the more complex system (Al, Ga, In) (As, Sb, P).

8. *Chemical Characterization of Magnetic Materials at High Spatial Resolution*

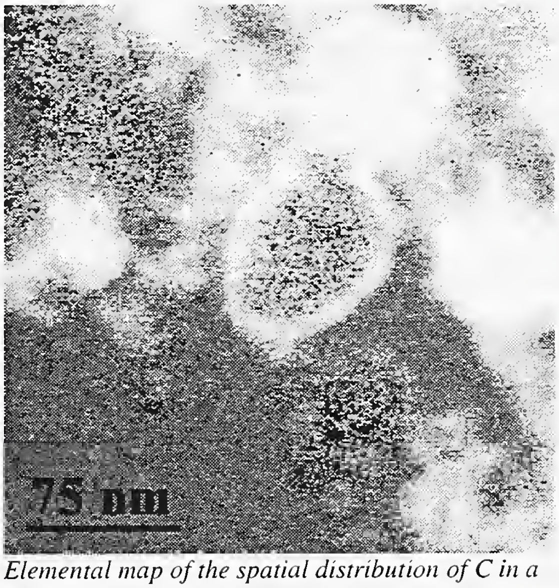
J.H.J. Scott

Objective: To adapt nanoscale chemical characterization techniques in the analytical electron microscope (AEM) to materials challenges presented by advanced magnetic nanocomposite samples.

Problem: Advances in magnetic materials research rely increasingly on information about chemical distributions at near-atomic dimensions. Sometimes this information concerns elemental distributions in artificial heterostructures, such as the multilayers in a spin valve. In other cases, information is needed about chemical or structural gradients that occur naturally during materials processing, such as recrystallization or segregation in an amorphous magnetic metal upon annealing. Several AEM-based nanoscale characterization techniques can be applied to these analysis problems, including convergent beam electron diffraction (CBED), energy-dispersive x-ray spectroscopy (EDS), and electron energy-loss spectroscopy (EELS). The Microanalysis Research Group has been applying these tools to magnetic nanocomposites and investigating next-generation techniques such as electron spectroscopic imaging (ESI), including energy-filtered transmission electron microscopy (EFTEM) and spectrum imaging.

Approach: The AEM is capable of both imaging and chemical analysis with very high spatial resolution. In this case, an intermediate-voltage transmission electron microscope/scanning transmission electron microscope (TEM/STEM) is used in TEM mode to image samples with a point-to-point resolution better than 0.2 nm, and is used in STEM mode to interrogate the sample with a finely focused probe approximately 1 nm in diameter. Conventional electron beam microanalysis is made possible by an EDS detector and an EELS spectrometer. Powerful new techniques have also been enabled by the addition of an imaging energy filter and a hyperspectral data acquisition system, designed to acquire EDS and EELS spectra simultaneously at each point of a 2-dimensional field of interest. Using the imaging energy filter, a series of energy-selected images acquired above and below a core-loss ionization edge can be combined to produce an elemental map of the sample, providing important clues to the variations in magnetic properties at very fine length scales. Multiple-energy-window maps can also be adapted to study changes in valence state of transition metal ions (*e.g.* Mn^{3+} vs. Mn^{4+}) in metals and magnetic oxides. Using hyperspectral data acquisition techniques and a scanned fine electron probe, both an EDS and an EELS spectrum can be associated with each pixel in a 1-dimensional profile or a 2-dimensional map. This "data cube" can then be processed offline to extract information such as compositional maps or valence state maps.

Results and Future Plans: The novel techniques described above were applied to magnetic nanocomposite powders and magnetic heterostructures. Sm-Co-C nanocomposites produced in an RF plasma torch from metal powder precursors and acetylene gas were mapped using EELS and energy-filtered imaging. Shown below is an elemental map produced using a 3-window electron spectroscopic imaging technique and energy-selected images acquired in the neighborhood of the carbon K-edge at 284 eV. Investigations of multilayered samples for spin valves and advanced spintronic devices are underway, and we currently have samples of melt-spun amorphous magnetic tapes and nanocrystalline soft magnetic materials such as $Fe_{44}Co_{44}Zr_7B_4Cu$.



Elemental map of the spatial distribution of C in a Sm-Co-C magnetic nanocomposite.

9. *Effect of AlN Encapsulation for High Temperature Annealing of Ion-Implanted SiC as Characterized by SIMS*

P.H. Chi, E. Handy and M.V. Rao (G. Mason Univ.)

Objective: To test an AlN thin film for encapsulating ion-implanted SiC prior to high temperature annealing. The film is intended to prevent the ion-implanted species from diffusing toward the surface.

Problem: SiC has been intensively studied due to its chemical and thermal stability and its wide band-gap characteristics. These characteristics make this material attractive for high power, high speed, and high temperature device applications. Recently, the ion implantation technique has been used to introduce dopants into SiC crystals at room temperature. Post-implantation annealing is needed to repair the crystalline damage and to activate the implanted species. However, the high temperature annealing also causes the implant to redistribute.

Approach: In this work, AlN was used as an encapsulating layer for As- and Sb-implanted 6H-SiC samples before high temperature annealing. These samples were annealed for 15 min. at 1600 °C and the profiles were characterized by secondary ion mass spectrometry (SIMS) to verify the effectiveness of the AlN encapsulating layers.

Results and Future Plans: Figure 1 shows the As depth profiles in the samples before and after annealing at 1600 °C for 15 min., with and without the AlN encapsulating layer. The main features of the arsenic profile before and after annealing with the AlN cap were that both samples showed a flat peak and a trailing profile tail which were the result of the multiple energy implant and ion channeling during ion implantation. The similarity of arsenic profiles in the As-implanted and annealed, capped samples reveals the effectiveness of the AlN cap layer in preventing arsenic diffusion under this annealing condition. For As-implanted SiC annealed without the AlN cap, As diffused toward the surface and more than 60 % of the As dose was lost to diffusion. The Sb profiles in SiC, annealed with and without the AlN protective cap, are shown in Figure 2. As observed for the As-implanted SiC sample, the Sb depth profile was quite stable at 1600 °C when annealed for 15 min. with the AlN encapsulating layer. Although the Sb tail was slightly broadened compared to the Sb profile before annealing, overall there was no major Sb redistribution in the annealed sample with the AlN cap. For Sb-implanted SiC annealed at 1600 °C for 15 min. without the AlN cap, Sb diffused toward the surface, with more than 90 % of the original Sb dose lost to annealing. The dopant loss mechanism can be understood by comparing the As and Sb SIMS profiles of the annealed samples with those of the samples annealed without an AlN encapsulating layer. The As and Sb losses were accounted for by sublimation loss of ~ 0.08 µm of the SiC surface during the 1600 °C/15 min annealing. As a layer of SiC sublimed, it carried with it the dopant within the layer. An AlN cap during annealing served to prevent the sublimation of SiC, and hence the loss of the dopant.

Future investigations will concentrate on using this AlN encapsulating layer on ion-implanted GaSb prior to high temperature annealing.

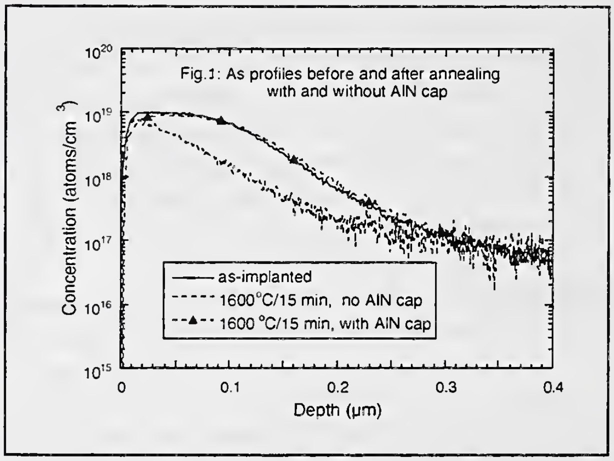


Fig.1: As profiles before and after annealing with and without AlN cap

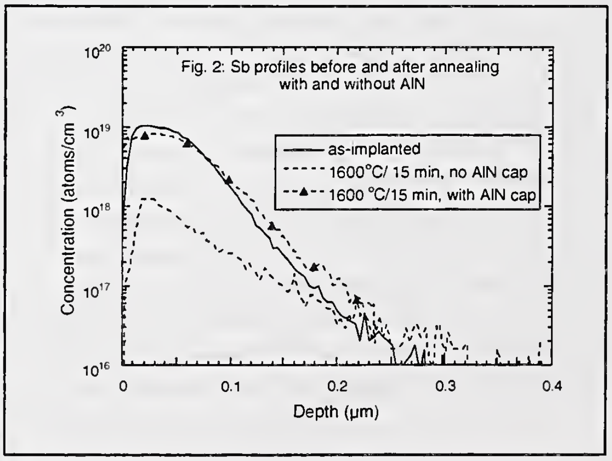


Fig. 2: Sb profiles before and after annealing with and without AlN

10. *Standard Test Data for Comparison of Curve-Fitting Approaches in Spectral Data Analysis*

***J.M. Conny** and C.J. Powell*

Objective: To evaluate curve-fitting approaches that analysts commonly employ by determining which approaches provide the best accuracy and precision in quantifying overlapping peaks from the set of simulated X-ray photoelectron C 1s spectra developed at NIST known as standard test data (XPS-STD).

Problem: The least-squares fitting of non-linear functions, in particular Gaussian (G) and Lorentzian (L) functions, is widely used to quantify spectral peaks. In addition to differences in how analysts set end points and model the baseline, there are several functions or ways to combine functions from which analysts may choose when fitting peaks. In XPS, analysts may employ a symmetrical G or a combined G-L function to fit individual peaks that appear symmetrical. The G and L components are typically combined into one function as a sum or as a product. Analysts commonly use a G-L function with an asymmetric tail, which is on the high binding-energy side of the peak. Analysts may also fit two non-linear functions to an individual peak to account for peak asymmetry. The success of a curve-fitting approach in terms of accuracy and precision depends on the spectral condition, *e.g.*, the extent and nature of asymmetry in the peak and the degree of overlap if more than one peak is present.

Approach: The XPS-STD set developed at NIST consists of 220 simulated polymer C 1s spectra in randomized order. 180 spectra were doublets with three levels of peak overlap, three configurations of the intensities between peaks, and two levels of Poisson noise. 40 spectra were singlets. Individual peaks exhibited very little asymmetry and baseline variation. Twenty analysts from industry, academia, and government determined peak energies and intensities using the following approaches:

1. single G function;
2. two G functions fit to each peak;
3. single asymmetric G function;
4. single G-L function;
5. two G-L functions fit to each peak;
6. single asymmetric G-L function; and
7. Voigt function.

Bias and random error were determined in the analysts' results. Bias was analyzed statistically with two non-parametric tests. The Kruskal-Wallis test, an analysis of variance based on rank, determined if the various curve-fitting approaches produced statistically different results for peak energies and intensities. The Man-Whitney U-test, also based on rank, determined which curve-fitting approaches produced the most accurate and precise peak energies and intensities.

Results and Future Plans: Even though peaks in the XPS-STD appeared highly symmetrical, the most accurate peak-energy measurements were with G-L functions that accommodated peak asymmetry. This included the use of dual G-L functions to fit individual peaks. For the doublets with the most peak overlap and with the larger peak at the higher binding energy, use of dual G-L functions produced better accuracy than the use of a single asymmetric G-L function. For this particular type of spectrum, relatively large negative biases in peak intensity were correlated significantly with relatively large positive biases in peak energy. This intensity-energy correlation suggests that one might consistently fit the component peaks of this doublet incorrectly but still produce an 'acceptable' fit based on, for example, minimizing chi-square.

Publication:

Conny, J.M., Powell, C.J., and Currie, L.A., ***"Standard Test Data for Estimating Peak-Parameter Errors in X-Ray Photoelectron Spectroscopy. I. Peak Binding Energies,"*** Surf. Interface Anal., 26, 939 (1998).

11. *Development and Application of Bootstrapping Methods for Analysis of Proficiency Test Data*

S. Turner, A. Au (Raytheon), M. Vangel, S. Leigh, A. Rukhin (898), E. Steel

Objective: To develop new methods for evaluating the performance of laboratories analyzing low-concentration proficiency test materials.

Problem: Over the past decade, the National Voluntary Laboratory Accreditation Program (NVLAP) has conducted proficiency testing of approximately fifty to one hundred laboratories involved in the analysis of asbestos by transmission electron microscopy. Since 1995, the laboratories have been sent quarter filter sections with asbestos deposited on them. It has been of interest to evaluate laboratory performance quantitatively over several proficiency tests. Such an evaluation is challenging for several reasons. First, the concentrations reported on these filters are near the detection limit of the technique. Since TEM analysis is very labor intensive, the data is very noisy with "counts" commonly on the order of 5-20 fibers per filter analysis. Second, the underlying distribution of data from filters has proven not to conform to an expected Poisson distribution. This may relate to the counting rules for asbestos analysis and to operator bias. Other challenges include incomplete data sets due to sample preparation problems or due to laboratories joining or leaving the testing program during the evaluation period.

Approach: To evaluate quantitatively the laboratories over several proficiency tests, a nonparametric (distribution-free) method was developed that uses a bootstrapping process. Bootstrapping is a computer-intensive, data-resampling method first proposed twenty years ago. It involves sampling of a data set with replacement to determine the probability distribution for a test statistic.

For characterization of the asbestos laboratories, proficiency test data collected over six rounds of testing is evaluated by comparison to other laboratories that analyzed the same filters. Three test statistics are determined for each analysis from each filter – the rank, percent difference from the filter mean, and the absolute percent difference from the filter mean. The average values for these test statistics are determined for each laboratory. Bootstrapping is used to determine probability distributions for each average statistic. For example, in the case of ranking, four analyses from a filter are ordered from highest to lowest concentration. The analyses are assigned a rank from one to four. The ranks obtained by each laboratory over six proficiency tests (typically 14 analyses) are averaged. Then, all ranks obtained by all laboratories are combined into a data set. Probability distributions are created by randomly choosing ranks from the data set. The number of ranks chosen corresponds to the number of analyses performed by the laboratories. The randomly chosen set of ranks is averaged and stored. This is repeated over one hundred thousand times to create a large set of possible ranks. This set is ordered from lowest to highest to form the probability distribution for the average rank. The probability or p-value for an average rank obtained by a laboratory is determined from its position in the bootstrapped data set. The determination of p-values for average percent difference and average absolute percent difference is done in a similar manner. For these cases, however, bootstrapping is done separately on several data sets corresponding to different filter concentrations deposited on the filters.

Results and Future Plans: This approach to analysis of proficiency test data has been used to evaluate laboratories in the last three rounds of testing. Laboratories are given the p-value for the three test statistics. Laboratories with outlier counts (consistently counting higher or lower than other laboratories) have been flagged. In the future, variations of this approach may be tried in which different test statistics are chosen.

12. *Role of Uncertainties Associated with Fundamental Parameters and Surface/Interface Gradation in Model Grazing Incident X-ray Photoelectron Spectroscopy Calculations*

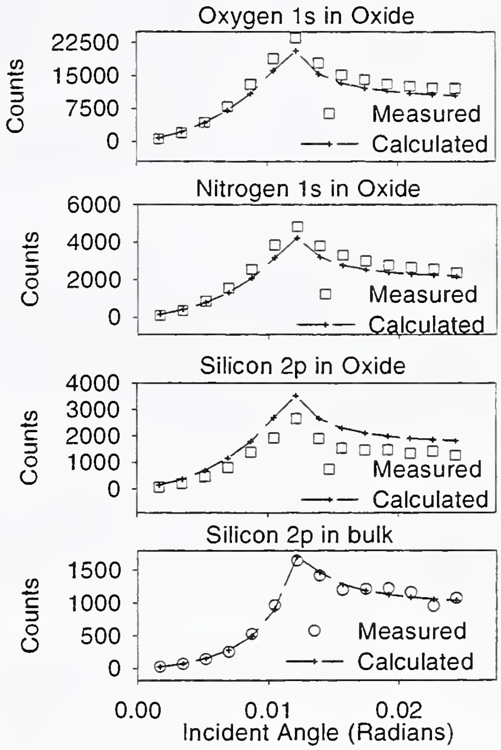
E. Landree

Objective: Requirements established by the National Technology Roadmap for Semiconductors have dictated the need for a metrological technique capable of characterizing thin oxide and dielectric films that are on the order of nanometers thick. A promising technique that is both non-destructive and sensitive to structure and local chemistry is grazing incidence x-ray photoelectron spectroscopy (GIXPS). Utilizing the non-linear dependence of photoelectron yield on quantities such as film thickness, density, cross-section, and x-ray index of refraction, it is possible to characterize a given layer structure by fitting these parameters to photoemission peaks measured for varying angles of x-ray incidence upon the surface, see at the right.

Problem: The fundamental material parameters, such as cross-section and index of refraction, are not well known, particularly within the energy range of interest (1-2 keV). In addition the effect of interface/surface abruptness upon the photoemission yield has yet to be characterized. Each of these parameters will influence the perceived film thickness and density.

Approach: An amorphous-carbon/silicon oxide/silicon substrate model is constructed and the electron photoemission is calculated using values for the ionization cross-section and index of refraction that differ from their nominal values. The deviation in perceived film thickness and density relative to the constructed model is then characterized by fitting these parameters while assuming the nominal values for the ionization cross-section and index of refraction. Similarly, the photoemission yield is calculated for models that include varying degrees of interface/surface gradation. The film thickness and density are then fit to the simulated photoemission yield assuming an abrupt atomic interface in order to observe the influence of gradation upon the perceived thickness and density.

Results and Future Plans:
Results indicate deviations in the index of refraction have a more dramatic effect on the perceived film thickness and density than corresponding deviations in the ionization cross-section. In addition, moderate interface/surface gradation produced only small deviations in the perceived film thickness and density. A comparison of this method, along with other techniques on an assortment of samples, should allow us to arrive at a set of acceptable values for the fundamental parameters that will be used in future analysis.



Photoemission yield for silicon oxynitride thin film

13. *New Databases for Surface Analysis by Auger-Electron Spectroscopy and X-Ray Photoelectron Spectroscopy*

*C. J. **Powell**, A. Y. Lee, D. M. Blakeslee, J. R. Rumble, Jr. (SRDP), A. Naumkin, A. Kraut-Vass, A. Jablonski, and W. S. M. Werner*

Objective: To provide needed reference data for surface analyses by Auger-electron spectroscopy (AES) and X-Ray photoelectron spectroscopy (XPS).

Problem: Surface properties are crucial for the fabrication and performance of a wide range of materials, semiconductor devices, optoelectronic materials, high-density magnetic-storage media, sensors, catalysts, thin films, and coatings. Surface analysis is used not only to correlate the composition of an exposed surface with specific material properties but also for the investigation of interface properties. AES and XPS are the two most commonly used techniques for surface analysis, and reference data are needed to improve the reliability and efficiency of surface analyses made by these techniques.

Approach: NIST established a Surface Analysis Data Center in 1997 to give greater visibility to its existing and planned databases for applications in surface analysis. The development of databases is carried out with the NIST Standard Reference Data Program and with the assistance of contractors.

Results and Future Plans: Three databases are now available for AES and XPS applications, and the development of another has commenced. These databases will be described in turn.

Version 1.0 of the X-Ray Photoelectron Spectroscopy database (SRD 20) was released in 1989, and version 2.0 was released in 1997. These databases have been widely used to obtain binding-energy and related data for elemental and chemical-state analysis of a wide range of materials by XPS. Version 3.0, to be released in early 2000, will be available for on-line access through the internet and will be free. It will contain a substantial amount of new data and additional information about the specimen material, the measurement conditions, and the data-analysis procedure for each reported measurement. The internet version has been redesigned to facilitate access, searches, and convenience. One feature will enable users to retrieve data by scientific citation. In this way, individual authors will be able to access their own (or others') data and will be able to check database entries. Work is ongoing to provide additional evaluated data for this database.

Version 1.0 of the Elastic-Electron-Scattering Cross-Section Database (SRD 64) was released in 1996. This database provides differential and total elastic-electron-scattering cross sections for elements with atomic numbers from 1 to 96 and for electron energies between 50 and 9,999 eV. It can be used for simulations of signal-electron transport in XPS and AES (and for other applications involving electron transport). Version 2.0, to be released in 2000, provides transport cross sections (needed for corrections of elastic-scattering effects in AES and XPS) and phase shifts (needed in some simulation codes); in addition, cross sections and phase shifts are available for electron energies up to 20,000 eV.

Version 1.0 of the Electron Inelastic-Mean-Free-Path Database (SRD 71) was released in September, 1999. This database provides calculated and measured electron inelastic mean free paths (IMFPs) for elements, inorganic compounds, and organic compounds. If no calculated or measured IMFPs are available for a material of interest, values can be estimated from two predictive formulae. IMFPs are needed for quantitative analyses by AES and XPS (corrections for matrix effects), determination of effective attenuation lengths (measurement of film thicknesses), determination of mean escape depths (average depths of analysis), and for simulations of the transport of signal electrons.

Work has commenced on a new database to be used for AES and XPS analyses of materials with complex morphologies. SRD 64 and SRD 71 will be combined with additional data to enable comparisons of measured and simulated spectra for particular specimen morphologies and specified analytical conditions.

14. *Isotopic Characterization of CO_2 from Industrial Sources: Infrastructure for Assessment of Reference Materials and Improved Traceability*

D.B. Klinedinst, *J. Lee, and R.M. Verkouteren*

Objective: To enable U.S. industry to realize high-accuracy isotope ratio measurements by developing a relevant suite of isotopic reference materials and associated Web-based interactive data tools to be used for calibration and normalization procedures.

Problem: The use of ^{13}C and ^{18}O high precision isotope ratio mass spectrometry (IRMS) has successfully been applied to high dollar impact industrial and environmental problems such as product authentication and groundwater pollution remediation. However, even with state-of-the-art measurement precision of about 0.03 ‰, achieving high accuracy $\delta^{13}C$ and $\delta^{18}O$ measurements has been elusive, which has limited many industrial and environmental applications of this technique. One of the primary factors contributing to this situation is the methodology employed to prepare the primary standard. By international consensus, $\delta^{13}C$ measurements are reported relative to the VPDB scale that has assigned values based on measurements of the primary standard artifact RM 8544, a carbonate material. $\delta^{13}C$ IRMS requires generation and measurement of CO_2 gas. Despite years of debate within the working community, an accepted standardized procedure for the conversion of the RM carbonate to CO_2 is lacking, and methods for the conversion, standardization and normalization of CO_2 isotopic measurement data to $\delta^{13}C$ values are inconsistent across laboratories.

Approach: We are developing the infrastructural tools (RMs and Web-based data routines) for utilizing CO_2 proxies rather than carbonate standards to realize the international isotopic scales for carbon and oxygen. Three CO_2 isotopic RMs that span the natural range of $\delta^{13}C$ compositions were prepared and characterized in our laboratory, the value and uncertainty assignments of which were determined by international consensus and unique measurement capabilities at NIST. Since wide compositional (^{13}C and ^{18}O abundance) gaps existed between these materials, we contacted several large providers and industrial manufacturers of CO_2 to help fill these gaps. Each manufacturer sent a cylinder of their liquefied product, and we analyzed samples for purity using self-consistent high accuracy measurement procedures developed in our laboratory, and provided feedback regarding the isotopic composition. We are also developing a Web-based *interactive standard reference data reduction algorithm* for use with the RMs. This algorithm (at http://www.acg.nist.gov/outputs/algorithm.html) permits an internet user to input measurement data of samples and RMs in a particular format that allows consistent data reduction, standardization, and normalization. The isotopic results can be calculated and returned to the user using a well-tested routine.

Results and Future Plans: We have completed our initial characterization of a suite of gases consisting of CO_2 derived from the following: (1) a polymer fiber manufacturing plant, where byproduct acid is neutralized, (2) a subterranean CO_2 archive, used by dry ice manufacturers and oil companies for petroleum recovery, (3) C_4 plants used for fermentation in a distillery, (4) C_3 plants used for fermentation in a brewery, and (5) the co-generation of H_2 and CO_2 associated with steam refining of petrochemicals. All but one of these CO_2 sources were of adequate purity to be considered candidate RMs. The standard reference data reduction algorithm was written in C++ programming code, compiled onto a Unix workstation, tested, and integrated into our Website. This algorithm will continually be improved as Web tools and internet standards improve. The combination of standard materials and Web-based data routines provides a powerful infrastructural tool for assuring data quality. Through this mechanism, traceability of CO_2 measurements to the international isotopic scales is improved by a factor of four, as measured by an international comparison exercise.

Publication:

Verkouteren, R.M., ***"Preparation, Characterization, and Value Assignment of Carbon Dioxide Isotopic Reference Materials: RMs 8562, 8563 and 8564,"*** Analytical Chemistry, 71, 4740 (1999).

15. *Multiple Electron Processes in Hot-Electron Femtochemistry at Surfaces*

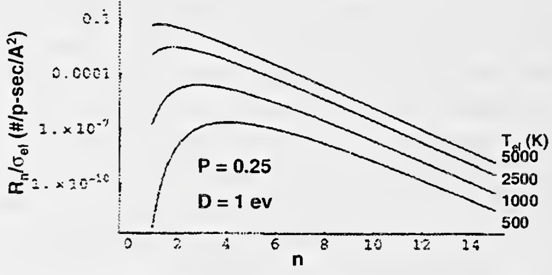
J. W. Gadzuk

Objective: To understand and theoretically quantify the role and multiplicity of inelastic hot-electron scattering events required for elementary chemical processing in laser-excited surface femtochemistry (SF).

Problem: Inelastic resonance scattering of femtosecond-laser-excited hot electrons in solids is the fundamental mechanism underlying many electron-stimulated processes such as desorption or dissociation of adsorbed molecules. Theoretical reaction rates or yields have been derived as an integral product of the energy distribution of the flux of incident hot electrons multiplied by the inelastic resonance electron scattering cross section. For fixed laser fluence, the temporally narrowest laser pulses produce the hottest electrons. The flux of laser-generated hot electrons incident upon the surface is given by an electron-temperature-dependent expression similar to the Richardson equation for thermionic emission. There is substantial experimental evidence that for the ~20 fs – 200 fs pulses used in state-of-the-art SF studies, the induced chemistry is due to multiple photon (hence electron) excitation. Existing "standard" theory suggests that the multiplicity should be significantly greater than ten. In contrast, we have shown that this is inconsistent with certain stringent statistical mechanics requirements thereby creating internal contradictions in our understanding of SF.

Approach: The transient response (such as a desorption rate or yield) of a surface-adsorbate system to a fs laser pulse has been obtained as a Master Equation rate for first passage into the desorption continuum after femtosecond-laser-heating of the substrate conduction band electrons to a temperature T_{el}. The desorption rate for breaking a bond of energy D via a sequence of n inelastic electron scattering events is $R_n = \sigma_{el}\, p^n\, j_n(T_{el})$ where σ_{el} is the electron capture cross-section, $p \cong 0.25$ is the probability per electron-adsorbate collision that energy D/n is delivered to the bond, and $j_n(T_{el}) = A\, T_{el}^2 \exp(-D/nkT_{el})$ is the "thermionic" hot electron flux of useful electrons upon the surface, with $A = 120$ amps/cm^2 and T_{el} given in K.

Results and Future Plans: For a given T_{el} , the incident flux of energetically useful electrons increases with increasing multiplicity n, whereas since p is necessarily less than unity, p^n , the total probability for an nth order process, must approach zero as n increases. Due to the opposing n-dependences of the cross section ($\sigma_{el}\, p^n$) and hot-electron flux, the optimal multiplicity is determined by a compromise, as illustrated below where the partial desorption rate R_n (with D = 1 eV, as for NO/Pt) is shown as a function of n, for several electron temperatures characteristic of SF. The strong dependence of the desorption rate on the electron temperature is evident. Furthermore, these results clearly show that SF is dominated by few-n processes, in contradiction to the widely used theory. This finding demands that a more appropriate general theory be devised for this important area in surface dynamics. Future plans include developing and extending our theory beyond the first-passage time domain, including realistic intra-molecular dynamics into the description of individual scattering events, and applying the theory to experimental realizations of unusual importance.



Partial desorption rate as a function of scattering multiplicity for a range of electron temperatures, as labeled.

16. *Oxygen Atom Reactions with Well-Characterized Surface Adlayers on Si(100)*

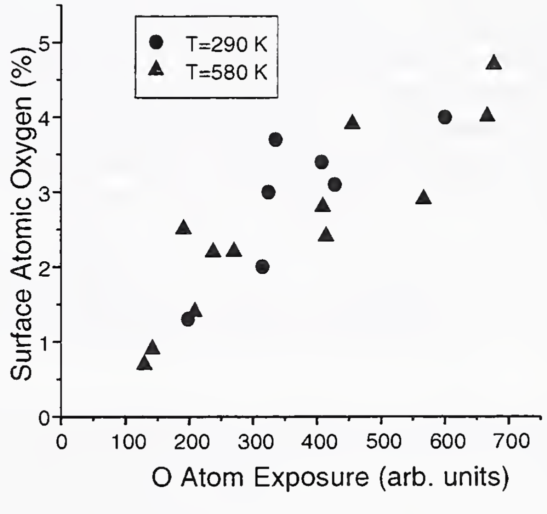
M. Litorja, L.M. Struck, and ***S.A. Buntin***

Objective: To quantitatively measure reaction probabilities and mechanistic details for radicals reacting with well-characterized surfaces of industrial relevance. Specifically, use a novel radical beam source to characterize the interactions of ground and electronically excited O atoms with adsorbate-covered Si(100).

Problem: For microelectronic device fabrication, a more quantitative understanding of the surface chemistry of semiconductors is necessary as the critical dimension of device features continues to shrink and the cost of empirical process optimization continues to rise. Industry-based strategic planning for the continued development of microelectronic processing clearly highlights an increased reliance on modeling and simulation for process optimization. However, it is recognized that there is a significant deficiency in our current knowledge of radical/surface interactions; there is a critical need to elucidate mechanisms and quantify probabilities for radical/surface reactions to meet roadmap goals and next-generation demands. In device fabrication, oxygen plasmas are often used to remove photoresist, and plasma-enhanced processes involving Si/O/H-based precursors are used for silicon dioxide deposition. For these applications, it is known that O atoms are key in the surface chemistry, but reaction rates and mechanisms are not known. With the drive towards lower pressure and temperature plasmas, it is necessary to determine the influence of electronic excitation on the surface reactivity of O atoms. Dramatic reactivity differences between (^{1}D) and (^{3}P) O atoms have been documented for reactions with small molecules in the gas phase (the ^{1}D metastable, excited state is 1.97 eV above the ^{3}P ground state). The effects of electronically excited O atoms in surface reactions, however, have not been systematically addressed.

Approach: We have developed a laser-photolysis-based method of producing a relatively "clean" flux of atomic radicals. This source is uniquely suited for probing O atom reactions since it is capable of generating very well-defined incident O atom fluxes; that is, exclusively ground-state (^{3}P) O atoms are produced from the 193 nm photolysis of SO_2, while a 50/50 mix of (^{3}P) and (^{1}D) O atoms is produced by the 157 nm photolysis of O_2. Well-characterized, fully saturated surface adlayers of deuterium (D), acetylene (C_2H_2) or ethylene (C_2H_4) on Si(100) are prepared in an ultrahigh vacuum environment. These adlayers are then subjected to varying exposures from the O atom beam source and the evolution of the surface adlayer composition (*e.g.*, oxygen and carbon relative to silicon) is followed by Auger electron spectroscopy.

Results and Future Plans: Studies thus far have considered O atom surface oxidation using only the 157 nm photolysis of O_2. For the monodeuteride-terminated Si(100) surface, the data shown below indicates no difference in the oxidation rate for surface temperatures of 290 K and 580 K, indicating that the oxidation process is not activated by the surface energy. While O atoms exposure dependencies have not yet been fully determined for C_2H_2 and C_2H_4 adlayers, the results indicate that there is no significant difference in oxidation rate for C_2H_2-, C_2H_4-, and D-Si(100). In addition, the surface carbon does not appear to be diminished by the O atom oxidation. These results suggest that the oxidation occurs at sites other than those associated with the Si-dimer bond, perhaps by insertion in the Si back-bonds. Further characterization of these systems is planned, including an evaluation of the dependence of the initial deuterium coverage.



Surface oxygen uptake vs. exposure on D/Si(100) for surface temperatures of 290 K and 580 K.

17. *In-situ, Real Time Studies of Vesicle Fusion via Vibrationally-Resonant Sum-Frequency Generation*

L.J. Richter, T.P. Petralli-Mallow (831), A.L. Plant (831), K.A. Briggman (844) and J.C. Stephenson (844)

Objective: To demonstrate the applicability of vibrationally-resonant sum-frequency generation (VR-SFG) to the *in situ* study of kinetics at liquid/solid interfaces.

Problem: Both the molecular structure of and the chemical reactions that occur at interfaces are critical to industries as diverse as chemical processing, biosensing, and semiconductor electronics. Often it is essential to characterize interfaces in their native environment. For example, the physical structure and chemical function of biological membranes depends critically on interaction with the water that surrounds them *in-vivo*. Thus the study of biomembranes is difficult as it requires probes that are both sensitive and selective (*i.e.* discriminate against the bulk material). Of those probes with adequate interface specificity, few have adequate time resolution to allow the study of kinetics.

Approach: VR-SFG measurements were performed with a novel, broad-band sum-frequency generation system developed at NIST [Opt. Lett. **23**, 1594 (1998)] that allows rapid spectral acquisition. VR-SFG involves the nonlinear mixing of an IR photon, resonant with vibrations in the sample, with a visible photon to produce a new photon at the sum frequency. It is uniquely interface specific as it is symmetry forbidden in centrosymmetric media. However, the interface must be optically accessible. A commercially available microscopy chamber was modified to provide a 25 μm thick laminar flow across the substrate providing a thin solvent layer allowing penetration of the IR photons.

Results and Future Plans: Hybrid bilayer membranes (HBMs) are model constructs for biological membranes consisting of a phospholipid monolayer on a functionalized support. To demonstrate the utility of VR-SFG combined with a thin laminar flow cell, we have studied the kinetics of the formation of a HBM via the fusion of phospholipid (d13-DPPC) vesicles from a D_2O buffer solution onto a gold substrate functionalized by a self-assembled monolayer (SAM) of deuterated octadecanethiol (d-ODT). Shown in the figure are selected VR-SFG spectra from a time series recorded during the formation of the HBM. The kinetics of fusion are clearly within the time-resolution of the measurement. The three strong features at 2875 cm^{-1}, 2935 cm^{-1}, and 2965 cm^{-1} can be attributed to the terminal CH_3 groups of the DPPC acyl tails, oriented with the H's facing the d-ODT substrate. The absence of strong CH_2 features at 2850 cm^{-1} and 2920 cm^{-1} indicate that the acyl tails have few gauche defects. The uniform increase of the CH_3 features indicates that the DPPC monolayer develops via the growth of ordered islands. Quantitative analysis of the time evolution of the features indicates that the island growth can be described by Langmuir adsorption kinetics, suggesting that vesicles physisorbed on the islands do not participate in the adsorption processes. The newly developed capability of *in-situ* VR-SFG will be used to characterize the influence of both lipid chain structure and substrate functionalization on the kinetics of HBM formation. The knowledge gained should lead to a better understanding of cell membrane dynamics, and possibly lead to improved designs for HBM based sensors.

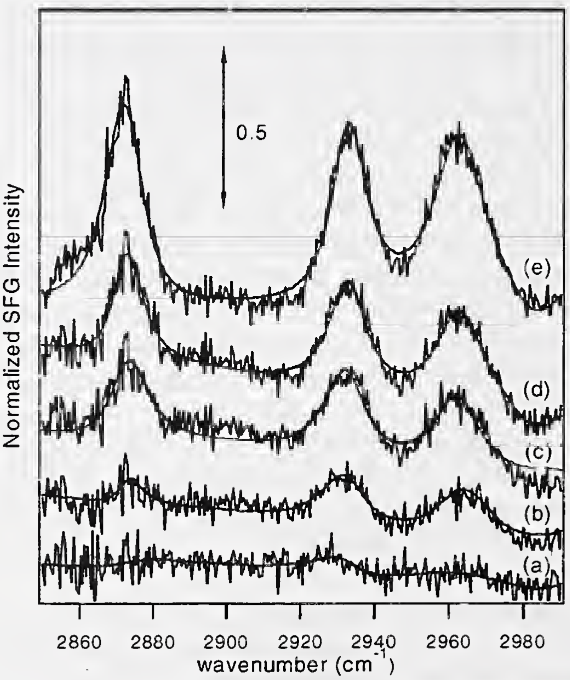


Figure 1. VR-SFG spectra collected at (a) 2 min, (b) 4 min, (c) 6 min, (d) 8 min, and (e) 34 min after the introduction of vesicles to the buffer solution.

18. *Isotopic Black Carbon in the Environment: New Metrology for ^{14}C and its International Impact*

L.A. Currie**, J.D. Kessler, and Contributors*

Objective: To develop highly reliable isotopic-chemical reference methods and reference materials for the apportionment of sources and the assessment of temporal and spatial distributions of black carbon (BC) in the global environment.

Problem: Black carbon, known also as elemental carbon and soot carbon, has long been one of the most elusive yet most critical species demanding high quality measurements and standards for the urban, regional, and global environments. The importance of fine particle BC derives from its unique role as a tracer of fire, combined with its impacts on visibility, health, and climate change. Isotopic speciation (^{13}C, ^{14}C) in BC is essential for the quantitative assignment of sources (apportionment), including anthropogenic which are subject to control, and natural, which are not. BC metrology is beset with twin problems: First, since BC is not a unique chemical substance, the resulting method dependence has led to many discordant results and erroneous conclusions. Second, since isotopic measurements require isolation of the BC often in minute amounts, conventional optical methods do not apply, and wet chemical methods may lead to severe problems with contamination and losses.

Approach: The first problem has been addressed through international comparison involving a multidisciplinary team and a broad range of chemical, thermal, and optical methods, all applied to the same NIST SRM 1649a (Urban Dust). The results of the comparison were presented at the Ninth Annual Goldschmidt Conference, and they are being incorporated in the new Certificate of Analysis for SRM 1649a. This represents the first comprehensive effort to provide "operational" (method-specific) BC Reference Values. The second problem has been attacked by developing a new "clean chemistry" method for BC analysis and isolation, the Thermal Optical Kinetic (TOK) technique. The TOK method, which was first introduced also at the Goldschmidt Conference, uses only high purity gases as reagents and a small quartz oven as reaction chamber. Both absorbance and carbon reaction rate are monitored with time, permitting the deconvolution of reactivity classes of components and sensing the onset of BC oxidation. By stopping the reaction at the appropriate time, minute BC residues can be isolated without losses and without reagent contamination. With TOK, valid high sensitivity ^{14}C accelerator mass spectrometry results have been obtained with as little as 6 µg BC, some 50 times smaller than that measured with (wet) chemical methods of isolation.

Results and Future Plans: The increasing importance of BC in the atmospheric, earth, and marine sciences was evident in the formation of the *International Steering Committee for the Development of Black Carbon Reference Materials* at the Goldschmidt Conference. This six-member committee, which includes NIST representation, has joint chairs at UC Irvine and the Max Planck Inst. für Biogeochemie, Jena. We expect it to serve as the central international vehicle for generation of suitable reference materials, method-specific reference values, and accuracy control through international comparisons. The TOK method shows great promise for high quality BC carbon isotopic data at the µg level. The small sample size, combined with the simultaneously produced chemical data, makes it an excellent candidate for objective and quantitative apportionment of atmospheric soot deriving from a variety of fossil and biomass combustion sources.

Publications:

L.A. Currie *et al.*, *Sympos. Black Carbon in the Environment*, paper 7149 (Aug. 1999).

L.A. Currie and J.D. Kessler, *Sympos. Black Carbon in the Environment*, paper 7148 (Aug. 1999).

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*Contributions from an international team of co-workers are represented in the *Black Carbon Steering Committee* and the *Black Carbon SRM 1649a Comparison Exercise.*

19. *Seasonal and Historical Records of Aerosol Carbon and ^{14}C in Greenland Snow and Ice: Initial Studies*

J.D. Kessler and L.A. Currie

Objective: To expand the capabilities of conventional accelerator mass spectrometry (AMS) and scanning electron microscopy (SEM) measurements to accommodate the need for synergistic measurement techniques capable of carbon quantification at the sub-microgram level.

Problem: The lack of sufficient and automated techniques for preparation, measurement, and evaluation of low carbon concentration (~1–10 ng/g) aerosol samples, accompanied by the increasing realization that black carbon (BC) contributes to global warming, has fueled the investigation to solve these problems. Characterization of these aerosols must be conducted on three levels: (1) mineralogical characterization on a particle-by-particle basis with SEM to fingerprint the BC origin(s); (2) separation of the BC fraction of the sample; (3) establishment of submicromole AMS techniques for measuring ^{14}C to distinguish between fossil and biomass burning sources of BC. Due to the vast quantities of geographical areas requiring these measurements, automated techniques are of interest.

Approach: All techniques and materials to achieve this objective were established at NIST and the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) Facility with the use of NIST SRM 1649a, 1515, and 2975 and various Greenland aerosol, snow, firn, and ice core samples. Automated data collection methods and a reusable boron substrate created at NIST have allowed rapid elemental quantification with $6 \leq Z \leq 30$ in the SEM using energy and wavelength-dispersive spectroscopy. Investigation of several BC separation methods led to the definitions of BC as an entity defined by its method of separation. The method of Thermal Optical Kinetics (TOK), developed by Currie, provided adequate automation and efficiency, while producing the lowest blank (<0.5 µg C) in comparison to the other BC separation methods. The implementation of the microsample combustion-dilution facility at NIST in conjunction with the NOSAMS small sample (25 µg) AMS target preparation facility have demonstrated successfully the modern carbon quantification limit (10 % rsd) of ~0.8 µg carbon.

Results and Future Plans: Three techniques are now established at NIST capable of preparing and measuring the carbon content of polar samples. Figure 1 illustrates the ability to measure the organic and black carbon species in a sample of ~5 µg carbon by TOK. Figure 2 illustrates an image and subsequent X-ray spectrum of a carbonaceous particle extracted from a 1996 Greenland snow pit. The initial results prove that quantification of carbonaceous species in remote air, snow, and ice samples is possible. The automated techniques to date encompass data collection, but are lacking in linking X-ray spectroscopy data to their appropriate aerosols and minerals. Following data collection and evaluation optimization, these techniques can be applied to any measurement field where the study of atmospheric aerosols is of interest.

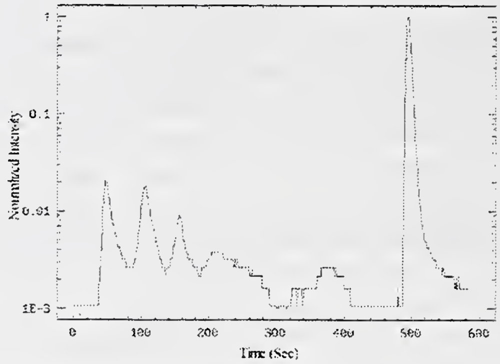


Figure 1 Thermal character of organic and black carbon from a filtered, 6.1 kg surface snow sample.

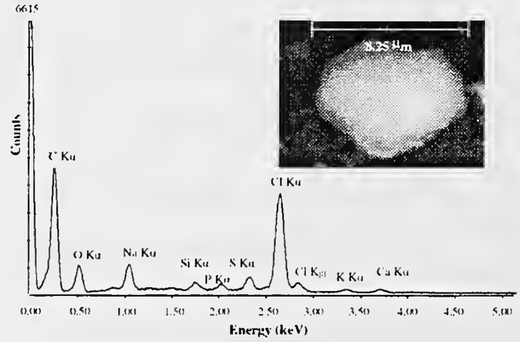


Figure 2 EDS spectrum and SEM image of a carbonaceous particle on a boron substrate extracted from a Greenland snow pit.

20. *Improved SIMS Methods for Detection of Enriched Uranium in IAEA Swipe Samples*

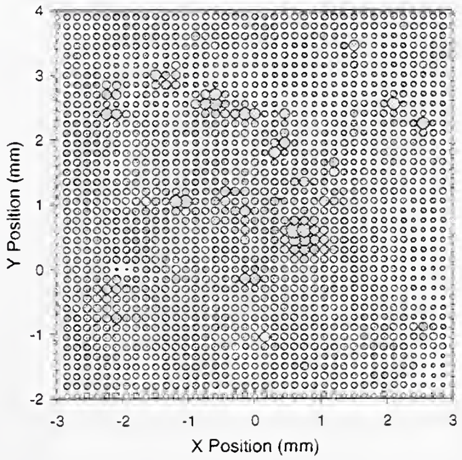
***D.S. Simons**, A.J. Fahey, J.G. Gillen, S.A. Wight, and C.J. Zeissler*

Objective: To develop procedures that improve the detectability and reliability of screening measurements for uranium by secondary ion mass spectrometry (SIMS).

Problem: For several years, the Analytical Microscopy Group has been developing and improving procedures to screen environmental swipe samples for the presence of enriched uranium. The driving force for this project is the mandate of the International Atomic Energy Agency (IAEA) to verify that signatories of the Treaty on the Non-Proliferation of Nuclear Weapons do not permit civilian nuclear materials to be diverted for military purposes. The IAEA conducts inspections of uranium enrichment plants and collects cloth swipe samples from them. These samples are analyzed to determine the isotopic composition of the uranium that is collected. We have developed a procedure based on SIMS to survey particulate matter extracted from the cloth swipe material and to measure the uranium isotopic composition from individual particles by a quantitative imaging method. In the course of analyzing samples from the IAEA, we have noted some analytical challenges related to the sparseness of uranium-bearing particles in some samples, and to molecular ions that produce spectral interferences with uranium in others. We have recently developed some new approaches to address these issues.

Approach: It would be useful to have a method to prescreen samples to decide whether enough uranium is present to make the SIMS analysis worthwhile. We have investigated the use of multi-day gamma spectral acquisitions and exposures to radiation-sensitive phosphor imaging plates as possible nondestructive screening methods applied to the cloth swipes. After particles are extracted from the cloth and deposited on a suitable substrate, the SIMS measurements are made. At this point, different procedures can be used depending on the density of uranium particles that are present. If the density is high, isotopic measurements can be made from each analytical area. This approach is time-consuming and usually covers only a few square millimeters of the substrate on which the particles are deposited. However, if the uranium density is low, it is more time-efficient to survey for only the major isotope of uranium (^{238}U), and return to those locations where uranium was detected for an isotopic analysis. In this way an area greater than 1 cm^2 can be surveyed in one day. We have noted that molecular ion interferences can perturb the apparent isotopic composition of uranium. In particular, ^{208}Pb^{27}Al and ^{207}Pb^{28}Si signals can be confused with ^{235}U and lead to a false detection of enriched uranium. We have found that the ratio of signals at m/z 234 to m/z 235 can be used as a diagnostic to indicate the presence of these spectral interferences.

Results and Future Plans: A comparison of low background gamma spectral acquisition and phosphor image plate exposure of test swipes for similar exposure times revealed that the phosphor imaging plate was able to detect activity from the cloth in several cases even though the gamma measurement was negative. The samples with positive detection were also found to have enough uranium to be suitable for SIMS measurements, indicating that the phosphor imaging plate can be a viable screening tool. The large area SIMS scanning method was applied to a sample of particles extracted from a cloth and dispersed on a graphite disk. A survey was made of an array of 6400 circular areas, each 150 µm diameter. The figure shows a bubble plot in which the log of the ^{238}U intensity is represented by the diameter of the bubble. The plot clearly shows the locations where high uranium signals were detected. Isotopic ratios were measured by SIMS from particles in these high-signal areas. In future work, this large area scanning method will be explored further to determine the minimum time in which a full sample can be surveyed.



21. *Removing Optical Artifacts in Near-Field Scanning Optical Microscopy*

S.J. Stranick, C. E. Jordan, L. J. Richter, and R. R. Cavanagh

Objective: To develop data acquisition and analysis methodologies for near-field scanning optical microscopy (NSOM) that distinguish and minimize artifacts while providing factual NSOM images.

Problem: In NSOM high spatial resolution is achieved by scanning a sub-wavelength aperture over the sample surface. Typically, the aperture is scanned while maintaining a constant gap (~ 3-5 nm) between the aperture and the sample producing a constant-gap mode (CGM) image. An NSOM based on CGM provides topographic and optical information. However, operating an NSOM in CGM on rough surfaces generates z-motion optical artifacts that arise from a change in optical intensity as the separation between the aperture and sample is varied. One method that eliminates z-motion artifacts is to acquire the data in constant-height mode (CHM) by scanning the aperture at a set height above the average surface plane. Direct acquisition of CHM images is hampered by the lack of suitable gap-maintaining technologies and prior knowledge of sample topography.

Approach: We have developed a method of acquiring NSOM data that allows for the construction of three types of images from one data set: topographic, CGM, and CHM. Instead of collecting optical data at a fixed aperture-sample gap at each XY position, optical data are collected along a controlled scan taken normal to the surface at each XY position. This produces cubes of data as shown in Figure 1a.

Results and Future Plans: The cube in Figure 1a shows the optical features of 80 nm gold particles immobilized on a silanized glass substrate. The cube covers a 1460 nm x 1460 nm x 140 nm region adjacent to the sample surface with topographic changes over this area of 90 nm. The grayscale represents the measured optical intensity. Z-motion artifacts are identified by analyzing the optical intensity for a given image as a function of the sample topography. The CGM image in Figure 1c shows many of the features present in the topographic image, Figure 1b. Z-motion artifacts often cause a direct correlation between optical and topographic features, and it is expected that many of the features in Fig. 1b are such artifacts. In the CHM image shown in Figure 1d the majority of the features observed in Figure 1c are no longer present, indicating that they resulted from z-motion artifacts. The prominent features observed for each particle in Figure 1d consist of a dark center spot surrounded by two lighter lobes. This is the factual NSOM image. We are currently developing an improved (reduced acquisition time) algorithm for the acquisition of artifact-free NSOM images as well as data analysis tools for evaluating CGM images for artifacts.

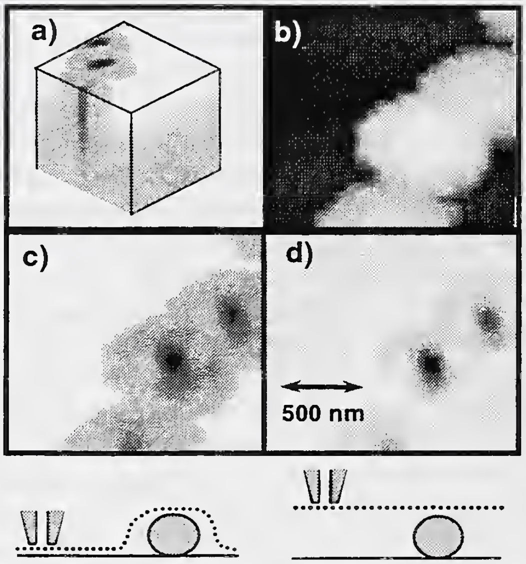


Figure 1. (a) Cube of NSOM data recorded in three spatial dimensions and one optical dimension. Images constructed from the (b) topographic, (c) CGM, and (d) CHM. The schematic underneath (c) and (d) indicate the path that a probe follows in CGM and CHM images, respectively.

22. *Near-Field Scanning Infrared Microscopy and Spectroscopy with a Broadband IR Laser Source*

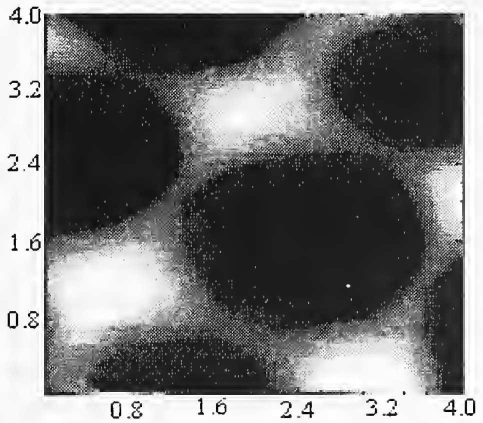
C. A. Michaels, L. J. Richter, S. J. Stranick, D. B. Chase (DuPont CR&D), and R. R. Cavanagh

Objective: To develop a novel optical probe for non-destructive, hyperspectral chemical imaging with nanoscale spatial resolution and chemical contrast based on infrared absorption spectroscopy.

Problem: Infrared absorption spectroscopy is a widely used technique for materials characterization as absorption in this spectral region depends sensitively on the molecular species present in the sample. Spatially resolved infrared absorption spectroscopy (microscopy) is likewise powerful and widely practiced yet the attainable spatial resolution (>20 µm) is limited by the range of available light sources and optics to well above the diffraction limit (~2-5 µm). Near-field microscopy yields optical images with spatial resolution finer than the limit set by diffraction; consequently this technique is evolving into an important analytical tool for the study of nanoscale material properties. There is great interest in an instrument that combines the chemical sensitivity of IR absorption spectroscopy with the high spatial resolution of near-field microscopy and generates images with chemical information on a sub-micrometer length scale.

Approach: A novel, benchtop infrared near-field scanning optical microscope (IR NSOM) has been developed, and the attainable spatial resolution and chemical imaging performance are being characterized. Key design elements of the microscope include: an ultrafast IR light source producing pulses with a FWHM bandwidth of 150 $\mathrm{cm^{-1}}$, a spectrograph based on an infrared focal plane array, and a near field probe fabricated from a single mode fluoride glass fiber. A novel chemical etching protocol for the fabrication of sub-wavelength apertures from fluoride glass fibers has been established. The parallel detection of the entire pulse bandwidth allows for the rapid acquisition of spectra (1 s) that is essential for true spectral imaging. A single map of a sample by the instrument provides both topography and absorption spectra over a strategically chosen band in the mid-IR.

Results and Future Plans: Transmittance images of several samples at 3.4 µm were used to benchmark the spatial resolution attainable with etched fluoride glass near-field probes. These samples include a thin film of TiO_2/polymer nanocomposite and a thin gold film patterned by microcontact printing. The figure shows a transmittance image of the patterned gold film. The dark regions are 10 nm thick gold. The light regions are bare silicon, which is nominally transparent at this wavelength. Cross sections through the narrowest region of silicon indicate spatial resolution of about 350 nm. These measurements validate the aperture fabrication methodology. This sample is now used routinely to characterize our near-field apertures. Near-field IR absorption spectra of a 750 nm thick acrylic melamine polymer film in the aliphatic C-H stretching region (3.3–3.6 µm) have also been acquired. These spectra match the far-field FTIR spectra and a 1 s spectral acquisition yields a signal-to-noise ratio of nominally 10:1 on a 4 % absorption feature. This confirms the instrumental capability of measuring high quality vibrational spectra of organic samples of sub-micrometer thickness with reasonable acquisition times. Spectral images of this sample were recorded for the C-H stretching region at every point in the image. Samples currently under investigation include phase segregated, thin polymer blend films (polystyrene/polybutadiene) obtained through collaborations with MSEL and BFRL. Spectral images in the aromatic C-H stretching region will be used to identify polystyrene rich domains thus allowing a stainless, non-destructive probe of polymer blend phase segregation.



A 4 x 4 µm near-field transmittance image at $\lambda = 3.4$ µm of a micropatterned thin gold film on silicon demonstrating spatial resolution of approximately $\lambda/10$.

23. *Carbon Cluster Primary Ion Beam SIMS for Organic and Semiconductor Surface Characterization*

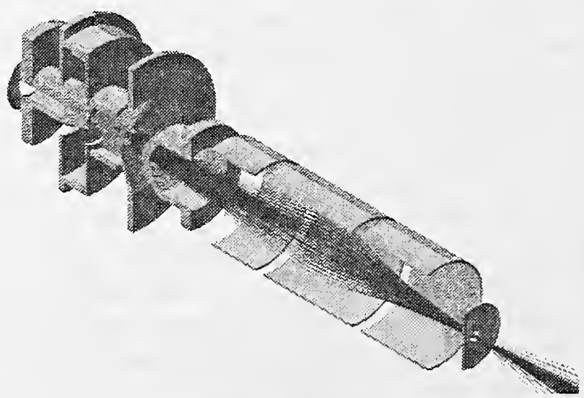
J.G. Gillen and B.D. Freibaum

Objective: To develop new approaches for secondary ion mass spectrometry (SIMS) using energetic cluster ion bombardment.

Problem: Secondary ion mass spectrometry (SIMS) is a surface analysis technique widely used for the chemical characterization of organic and semiconductor surfaces. The basis of the technique, as well as its fundamental limitation, is the requirement for bombardment of the material to be analyzed, in vacuum, with an energetic (keV) primary ion beam. This primary ion bombardment, typically with a species such as Ar^+, O_2^+, Ga^+ or Cs^+, produces the characteristic sputtered secondary ion signal that gives information on the surface and in-depth composition of the sample. Unfortunately, the bombardment process also results in extensive alteration of the near-surface region of the sample. The depth of this damaged or "altered layer" is directly related to the penetration depth of the primary ion and is largely responsible for determining the depth resolution of a SIMS depth profile. For organic surfaces, the creation of a subsurface damage layer results in the rapid degradation of the molecular structure of the surface, preventing the acquisition of molecular depth profiles and greatly reducing sensitivity.

Approach: The Surface and Microanalysis Science Division has become actively involved in the development and utilization of polyatomic and cluster primary ion beams for SIMS analysis. Because a cluster ion dissociates upon impact with a surface, the penetration depth of the constituents of the cluster are greatly reduced as compared to monoatomic primary ion bombardment under the same conditions. Furthermore, the simultaneous and temporally correlated impacts of multiple atoms from the cluster produce very large, non-linear enhancements in the number of atoms or molecules sputtered per cluster impact. We have demonstrated that the combination of these two effects can increase the yield of characteristic molecular secondary ions, more efficiently desorb higher molecular weight species, and reduce the accumulation of primary-beam-induced damage. For depth profiling of semiconductors, a cluster beam may offer substantial improvements in depth resolution. We are working with a small U.S. company (Peabody Scientific, Peabody, MA) to develop and explore the use of a negative cesium sputter ion source for generating carbon cluster ion beams for practical SIMS analysis.

Results and Future Plans: The figure shows a computer- generated model of the carbon cluster source currently being used at NIST. The cluster ions are produced by energetic Cs^+ bombardment of a graphite target in the source region. Primary ion beam currents of 1 μA of C_2^- have been produced. Cluster ions ranging from C_2^- to C_{10}^- are routinely used for analysis and depth profiling. For organic SIMS applications, the use of carbon cluster ions greatly increases the yield of molecular secondary ions. Under low primary ion dose bombardment conditions, the molecular ion yield from amino acid targets was found to increase by as much as a factor of ~800 when comparing C_1^- to C_8^- primary ions. Under high dose bombardment, the yield enhancement can be greater than 10,000. The larger carbon cluster ions also reduce the accumulation of beam-induced damage, allowing for sustained molecular ion emission at high primary ion doses. Semiconductor characterization has focused on examination of low energy As implants in silicon. Preliminary results suggest that the depth resolution obtained with a CsC_6^- cluster ion is improved by a factor of four as compared to conventional Cs^+ depth profiling. The source can also be operated in a microfocused mode, allowing micrometer spatial resolution images to be obtained. Current work includes further optimization of the source design and studies of the fundamental interactions of cluster ions with surfaces.



Sputter ion source.

V. Physical & Chemical Properties Division (838)

Robert E. Huie, Acting Chief

A. Division Overview

Mission

The Physical and Chemical Properties Division serves as the Nation's reference laboratory for measurements, standards, data, and models in the areas of thermophysics, thermochemistry, and chemical kinetics. The Division focuses primarily on:

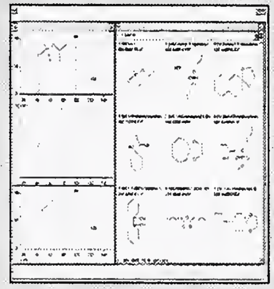
- thermochemical and thermophysical properties of gases, liquids, and solids, both as pure materials and as mixtures;
- rates and mechanisms of chemical reactions in the gas and liquid phases; and
- fluid-based physical processes and systems, including separations and low-temperature refrigeration and heat transfer.

The Division outputs include technical reports, Standard Reference Data, internet-based databases, calibrations, and Standard Reference Materials.

In order to carry out this Mission, the Division:

- develops, maintains, and utilizes advanced experimental tools and applies these to problems of scientific and industrial importance;
- compiles, evaluates, correlates, and interprets experimental data;
- develops and evaluates new theories, models, estimation methods, and computational algorithms;
- develops new dissemination mechanisms while maintaining a strong publication record in traditional media;
- carries out research leading to engineering data and models for advanced technologies; and

provides standards and services for fluid flow under cryogenic conditions.



Physical & Chemical Properties

R. Huie, Acting Chief

Fluid Science
Experimental Kinetics
& Thermodynamics
Chemical Reference
Data & Modeling
Computational
Chemistry
Experimental
Properties of Fluids
Theory & Modeling of
Fluids
Cryogenic
Technologies

Programs

The Physical and Chemical Properties Division is organized into seven groups:

- Fluid Science;
- Experimental Kinetics and Thermodynamics;
- Chemical Reference Data and Modeling;
- Computational Chemistry;
- Experimental Properties of Fluids;
- Theory and Modeling of Fluids, and
- Cryogenic Technologies.
-

The last three Groups are located in Boulder, CO, as are two Projects, Properties for Process Separations and Membrane Science and Technology. The activities in Boulder are under the general direction of William M. Haynes. The rest of the Division is located in Gaithersburg, MD. These Groups and Projects are engaged in a number of research activities, which arise from several focus areas that cut across Group/Project boundaries. These focus areas are discussed below, followed by selected Technical Reports from these activities.

Basic Reference Data

Industry is demanding reliable and accessible reference data on the physical and chemical properties of a wide variety of compounds at an increasing rate. These data are required in the development of models for process design, energy efficiency, and in the evaluation of possible environmental impacts. Basic reference data are also critical to the transportation and storage of fluids and in custody transfer.

The development of databases for use in industry and academia is a fundamental task of all the focus areas within the Division. Thus, many of the Technical Reports pertain both to Basic Reference Data and to a specific technical focus area. The notable exception to this is the NIST Chemical WebBook, which is a data dissemination tool that is designed ultimately to provide a link for most of our data activities (see Technical Report 1). Various data activities of the Division are discussed in Technical Reports 2, 5, 12, 15-18, 21-23, and 26. The strong integration of data activities with the experimental and computational programs means that experimental efforts often arise out of needs that these data activities identify. A recent example of this is the experimental and computational efforts that arose out of the project to create a focused database for chlorination chemistry (See Technical Report 11).

Computational Chemistry

The continued increase in computing power along with robust quantum mechanical codes is making the *ab initio* calculation of chemical properties an important tool for the industrial chemist. Progress in this area, however, is hampered by the lack of standards, comparisons, and simplified methodologies. In addition to this external need, the experimental projects within the Division benefit greatly by having a strong computational component. These considerations lead us to establish a computational chemistry focus area in the Division. Thus, we have initiated projects to compile, evaluate, and disseminate information about computational techniques (See Technical Report 2) and to develop improved methodologies for calculating thermodynamic and kinetics parameters (See Technical Reports 3, 4, and 20).

Future plans include an extension into condensed phase chemistry, which will utilize the Division's capabilities in molecular dynamics (see Technical Report 29) and will be coupled with experimental efforts in solution-phase and supercritical-water kinetics (See Technical Report 6).

Data for Process and Product Design

At some point in the manufacture of almost all the products that we use, there is a chemical transformation or separation process involved. In modern industry, the products and processes are designed and optimized by process simulators. These rely on fundamental physical and chemical property data. The Division's goal is to provide the data that industry needs to effectively apply process modeling and simulation at all appropriate points in the manufacturing cycle, from the separation and treatment of raw feedstock, through the manufacturing process, to the ultimate treatment and disposal of waste streams. This has led to a wide array of projects in the Division, some of which are strongly focused on a specific problem. Areas of current interest include:

- Properties of new solvents (See Technical Report 5)
- Solvation and reactions in supercritical media (See Technical Report 6)
- Membrane and other separation techniques (See Technical Reports 7-9)
- Semiconductor processing (See Technical Report 10)
- Chlorination chemistry (See Technical Report 11)
- Carbon dioxide conversion (See Technical Report 14)

In addition to providing the basic physical and chemical property data needed for process simulation, the Division is also active in the development and application of simulation techniques applied to both reacting chemical systems (See Technical Reports 12 and 13) and complex fluid systems (See Technical Reports 27 and 28).

Properties of Energy-Related Fluids

Energy-related fluids include both those which are primary sources of energy, the fuels, and those which interconvert heat and useful work – the working fluids. In several key areas, industry requires accurate and comprehensive equilibrium and transport property data and models for these fluids. These areas include the design and optimization of working cycles in refrigeration and power production systems; the design and control of gas processes; custody transfer; and in the development of new, cleaner energy systems. Efforts in the Division to meet these needs include the development of experimental apparatus for thermophysical property measurements; the acquisition of data; and the development and dissemination of accurate correlations. Other work has focused on the thermophysical and transport properties of mixtures of alternative refrigerants with lubricants. An important facet of this focus area has been participation in the development of internationally accepted standards. Examples of activities under study in this focus area include:

- Standards for refrigerant properties (See Technical Report 15)
- Natural gas systems (See Technical Report 16)
- Hydrogen-enriched fuel systems (See Technical Report 17)

- Refrigerant transport properties (See Technical Report 18)
- Petroleum fractions and refrigerant/lubricant systems (See Technical Report 26)

Environmental Fates of Industrial Chemicals

The use of chemicals in American industry is ubiquitous, and much of the Division's efforts go toward improving these processes. The fate and disposal of these industrial chemicals and the associated byproducts are also of great concern. A wide variety of physical and chemical data is essential to understand the fate and impact of industrial chemicals in the environment, to develop strategies for the removal or destruction of harmful byproducts, or to design processes and products which minimize environmental impact. One of the considerations for choosing new data sets for inclusion into the NIST WebBook is environmental importance. Thus, in the past year Henry's law constants were added along with many new vapor pressure values (see Technical Report 1). We have carried out studies on the atmospheric chemistry of industrial compounds for many years, spanning studies of reactive species which may contribute to photochemical smog, to much less reactive species which may contribute to ozone depletion or global warming. Providing fundamental data in support of chemical disposal technologies is a relatively new activity, although it was a driving factor behind the development of a supercritical water reactor (See Technical Report 6). Technical Report 19 discusses recent results from a collaborative effort in the application of our expertise in radiation chemistry to a serious waste disposal problem. Other work in the Division includes studies of the phase equilibria, coexisting densities, and interfacial tensions of mixed electrolyte/solvent waste streams.

During FY99, we initiated a new project to leverage our experimental capabilities in atmospheric chemistry by use of new capabilities in computational chemistry. This was prompted by studies that demonstrated the shortcomings of simple structure-activity relationships. The long-term objective of this project is to establish a theoretically justified means of predicting the atmospheric reactivity of new classes of compounds with the use of a limited number of selected experimental studies for verification. Progress in this area is summarized in Technical Report 20.

Tools for Chemical Analysis

Central to all of chemistry is the analysis of complex mixtures and the identification of the individual chemical constituents. These analyses are usually derived from basic physical/chemical properties of the species, and knowledge of these properties is thus critical to the reliability of the information. The Division strives to produce evaluated data, predictive algorithms, and analysis software to assist in the identification and quantification of a range of species under diverse conditions. The NIST WebBook plays a central role in this, but current Division activities also include measurements and data acquisition designed to expand the gas chromatographic and mass spectrometric databases (see Technical Reports 21 and 22). A critically important activity in the Division is the development of complex algorithms for the rapid and automatic analysis and deconvolution of GC/MS data for the identification of chemical-weapon agents (See Technical Report 23).

Fundamental Studies of Fluids

In support of the Division mission to provide U.S. industry with thermophysical properties of gases, liquids, and solids, the Division maintains a focus area on the fundamental studies of fluids, with strong experimental and theoretical components. The goals are to develop and utilize unique experimental, theoretical, and simulation capabilities to study fluid systems under equilibrium and nonequilibrium conditions. Much of the work relates to phase boundaries, vapor-liquid and solid-fluid equilibria, including complex interactions leading to gel formation. Some of the areas the Division is focusing on are:

- Surface tension of mixtures (See Technical Report 24)
- Solid-liquid equilibrium (See Technical Report 25)
- Thermophysical properties of partially characterized systems (See Technical Report 26)
- Relationship between fluid properties and shear (See Technical Report 27)
- Radiation scattering and simulation studies of complex fluid systems (See Technical Reports 9 and 28)
- Microheterogeneity in liquids (See Technical Report 29)

In selected cases, the measurements and the calculations of the thermophysical properties of gases have been refined to make fundamental contributions to metrology. We have used very accurate measurements of the speed of sound in argon between 200 K and 300 K to determine the differences between the internationally accepted temperature scale (ITS-90) and the Kelvin thermodynamic temperature. This work is being extended to 800 K in collaboration with the Temperature Group of Division 836 (see Technical Report 30). Our *ab initio* calculations of the thermal conductivity, viscosity, and second virial coefficient of helium are now more accurate than the measurements of these properties. Thus, calculated "data" can be used to calibrate instruments made to measure these properties. With a newly funded competence program, we are improving the measurement and the *ab initio* calculation of the dielectric constant of helium. Our goal is to use gas-filled capacitors to calibrate piston gauges in the range 0.5 MPa to 5 MPa.

Cryogenic Technologies

Cryogenic technologies are critical to a wide variety of technically and industrially important areas. These include the cooling of electronics for optical sensing and high-speed computing; the production of ultra-clean vacuum environments for semiconductor and other manufacturing processes; the liquefaction of natural gas and other industrial gases on demand; and in numerous medical applications. The research of the Division in this area involves the application of thermophysical concepts and measurements for temperatures below 120 K. This research has focused primarily on improved measurement and modeling techniques involved in the development and characterization of novel and improved cryocoolers (See Technical Report 31), studies of microscale heat transfer, and the maintenance and improvement of the national standard for cryogenic flow measurements (See Technical Report 32). As part of an upgrade, the cryogenic flow loop has been brought into compliance with ISO Guide 25 requirements.

Organizational Structure

Division-Office Projects (Gaithersburg and Boulder)

- Study the behavior of fluid systems under both equilibrium and nonequilibrium conditions using unique experimental, theoretical, and simulation capabilities.

Properties for Process Separations Project (Boulder)

- Performs research and provides critically-evaluated data and models on a variety of fluid-based separation processes, including distillation, adsorption, and supercritical fluid extraction.

Membrane Science and Technology Project (Boulder)

- Performs research on characterization techniques and provides fundamental data and models needed to design and/or select more efficient and robust materials for membrane-based separations.

Fluid Science Group (Gaithersburg)

- Develops and applies state-of-the-art techniques based on acoustics and other novel approaches for measuring the thermodynamic and transport properties of fluids and fluid mixtures, including refrigerants and semiconductor processing gases.
- Performs research on next-generation primary standards in the areas of temperature, pressure, and low flow rate.

Experimental Kinetics and Thermodynamics Group (Gaithersburg)

- Develops and uses state-of-the-art measurement techniques to determine the rates and mechanisms of chemical reactions in the gas and liquid phases and the thermodynamic properties of industrially and environmentally important chemical species and materials.
- Develops new measurement methods for detecting and characterizing reactive intermediates.
- Certifies Standard Reference Materials for thermodynamic properties important to industry and science.

Chemical Reference Data and Modeling Group (Gaithersburg)

- Develops and evaluates new theories, models, and estimation methods for thermodynamic properties, rate constants, and molecular spectra
- Compiles, evaluates, correlates, and disseminates Standard Reference Data.
- Develops and disseminates electronic databases and software on thermodynamics, chemical kinetics, and analytical mass and infrared spectra.

Computational Chemistry Group (Gaithersburg)

- Develops and applies computational methods for calculating the chemical and physical properties of selected species and systems.
- Critically compares computational predictions with the best available experimental data to establish the accuracy and reliability of computational methods.
- Develops resources to provide guidance to non-experts on methods, reliability, and resource requirements.

Experimental Properties of Fluids Group (Boulder)

- Performs experimental research and develops and maintains high-accuracy apparatus for measuring the full complement of thermodynamic and transport properties of fluids and fluids mixtures over wide ranges of temperature, pressure, and composition
- Provides comprehensive thermophysical property measurements for technically important pure fluids and mixtures, including common organics and inorganics, hydro-carbons, refrigerants, and aqueous systems.

Theory and Modeling of Fluids Group (Boulder)

- Performs theoretical and simulation research on the thermophysical properties of fluids and fluid mixtures, including regions of fluid-fluid and fluid-solid phase separation.
- Develops models and correlations of high accuracy to describe and predict the thermophysical properties of fluids and fluid mixtures.
- Provides comprehensive and evaluated Standard Reference Data and electronic databases for the properties of technically important fluids and fluid mixtures.

Cryogenic Technologies Group (Boulder)

- Develops improved measurement and modeling techniques for characterizing basic cryocooler components and processes.
- Develops prototype state-of-the-art cryocoolers for specific applications.
- Provides measurement standards and services for flow under cryogenic conditions.

Staff Recognition

- **Ray Radebaugh** was awarded the J&E Hall Gold Medal from the Institute of Refrigeration for research on the development and theory of pulse tube refrigerators.
- **Eric W. Lemmon, Mark O. McLinden, and Adele P. Peskin** (895) received an SRD Measurement Service Award for their contributions to the development of the RefProp Database.
- **Steven E. Stein** was selected to receive the Patterson-Crane Award by the Columbus and Dayton Sections of the American Chemical Society for contributions to chemical documentation.
- **Michael J. Kurylo** received a Certificate of Appreciation from the United Nations Environmental Programme for his contributions to the 1998 assessment activities conducted under the auspices of the Montreal Protocol.
- **Eric D. Marquardt and Ray Radebaugh** received the Russell B. Scott Memorial Award for the Outstanding Paper in Cryogenic Engineering Research, which was presented at the Cryogenic Engineering Conference.
- **Steven E. Stein** received the 1999 ANACHEM award from the Association of Analytical Chemists, for his outstanding research achievements and service to the field of analytical chemistry.
- **Michael R. Moldover** was recognized for presenting the best oral presentation at the Seventh International Symposium on Temperature and Thermal Measurements in Science and Industry.
- **Mark O. McLinden** was selected for the NIST Slichter Award for working closely with the air-conditioning/refrigeration industries to replace ozone-depleting CFCs with environ-mentally acceptable alternatives.
- **Joe W. Magee** was given the Diversity Award for the NIST-Boulder Laboratories.

- **Michael R. Moldover and Robert F. Berg** (836) were part of a team which received the NASA Lewis Distinguished Publication Award for their paper, "Equilibration Near the Liquid-Vapor Critical Point in Microgravity," Phys. Rev. E 57, 436 (1999).
- **Patrick A. G. O'Hare** (retired) was made a Fellow of IUPAC.
- **Jan V. Sengers** has been elected a Fellow of the American Institute of Chemical Engineers for his significant contributions to the chemical engineering community.

B. Selected Technical Reports

1. *The NIST WebBook: NIST Chemical Reference Data for Industry*

***W.G. Mallard,** P.J. Linstrom, J.F. Liebman (Univ. of Maryland, Baltimore County), and P.J. Christian*

Objective: To provide Internet access to a complete set of chemical data with a common interface that is both easy to use and easily expanded.

Problem: There is an enormous amount of organic thermochemical data (heats of formation, entropies, heat capacities, heats of reaction) that are largely unknown to the technical community. One part of this project is to find and evaluate those data. In addition, there is a need to make available ancillary thermochemical data such as phase-change enthalpies. Data on infrared, ultraviolet, and mass spectra are often difficult to find. For all of these data, it is essential that tools be developed to provide easy access.

Approach: It is clear that the World Wide Web has dramatically changed the way that scientific information is communicated. The use of the Web as a publishing medium and as a resource for communication has been growing rapidly. What has not followed is the development of reliable data resources for the Web. The WebBook is an effort to correct this. The WebBook provides a fast and direct source of data available at all times. The initial efforts have been tied to an approach based on compounds rather than properties. All the data on benzene, for example, are gathered together, rather than all the data on heat of combustion. While the major thrust of the WebBook is to supply data from NIST evaluations, the role of the WebBook in providing a resource for chemical data from all sources will expand. In parallel with the efforts to gather and evaluate data, another major part of this project is aimed at providing the mechanisms needed to make these and other NIST chemical reference data available on the Internet. These efforts are part of NIST's program on Systems Integration for Manufacturing Applications (SIMA).

Results and Future Plans: During FY99 the fifth edition of the NIST Chemistry WebBook* was released. The total number of compounds for which data are provided has steadily increased and in the fifth edition, data for more than 31,800 compounds are available. As with every previous release, new data types were added; specifically, Henry's law data and UV/Visible spectral data. In addition, new data on critical constants, vapor pressure, and ion energetics were added, as well as significant increases in many of the thermodynamic data types. A new tool for substructure searching using chemical structures drawn by the user has been added. The number and variety of users—in industry, government, and academia—are a clear indication of the need for this type of service. Between 6000 and 12000 users per week use the Chemistry WebBook, an increase of about 25% from last year; and the fraction of returning users is between 45% to 55%. The WebBook is also a tool to aid future evaluation projects both at NIST and in collaboration with others. The goal of this project is to have a single point of entry for access to all chemical data at NIST. It is anticipated that during FY 2000, there will be two releases of the NIST WebBook. Additional data as well as new searching software will be included.

*http://WebBook.nist.gov

Publications:

Linstrom, P.J. and Mallard, W.G., ***"The NIST WebBook: A Tool for Chemical Data Access on the Internet,"*** Proceedings of the 10th International Chemical Information Conference and Exhibition, Nimes, France, October 18-21, 1998.

Mallard, W.G., and Linstrom, P.J., eds., ***"The NIST Chemistry WebBook,"*** http:\\WebBook.nist.gov.

2. *Computational Chemistry Comparison and Benchmark Database*

R.D. Johnson III

Objective: (1) To provide a benchmark set of molecules and reactions for the evaluation of *ab initio* computational methods. (2) To allow the comparison between different *ab initio* computational methods and experiment for the prediction of thermochemical properties. This will allow accuracy in the computed thermochemical properties to be estimated.

Problem: As computer power increases, there is more reliance on modeling and computational chemistry in the chemical industry. This use is owed to the increased safety and speed, and decreased cost of models and calculations when compared with laboratory measurements. This trend will continue as the computers and software become more powerful. *Ab initio* computational chemistry methods can provide accurate values for structures, entropies, and heats of formation. However, the cost of the calculation increases greatly as the accuracy increases. The errors in the computational methods are systematic, depending on the method and functional groups that compose the molecule. In order to take advantage of the computational methods, accuracy and cost need to be evaluated. A set of test molecules is needed for this evaluation. There are small sets of species for comparing theory and experiment [L. A. Curtiss *et al.*, J. Chem. Phys. 109, 42 (1998)], but a recent workshop on Computational Thermochemistry at the ACS 212th National Meeting identified the need for a larger set of species, on the order of 500.

Approach: In order to facilitate testing, we selected a set of benchmark molecules and reactions with reliable thermochemical and spectral data and for which both the values and the uncertainties had been evaluated. These measured data include gas-phase enthalpies of formation, entropies, vibrational frequencies, and structures. In addition, we are generating data from *ab initio* calculations for comparison with experiment. The calculations cover eighteen methods using six basis sets.

The presentation will be through a Web interface where a user can select a subset of molecules from the database (*e.g.*, all species containing phosphorus or an NH_2 group), and the property for comparison (*e.g.*, heat of formation). The user will be presented the experimental and calculated values as a table or chart. By comparing the experimental and computed thermochemical values for a given set of molecules, the systematic errors in the computed values can be determined.

Results and Plans: A set of over 600 species with well-known enthalpies of formation has been assembled. Both experimental and calculated values are accessible over the web. Tools are being developed for viewing and accessing this data, such as comparing experimental or theoretical enthalpies of atomization at a given temperature, and comparing reaction energies for user-specified reactions. Experimental data are being collected and evaluated. *Ab initio* calculations are ongoing. In addition to heats of formation, structures and vibrational energy levels are being included. Beta testers are reviewing the database now. (Web site: http://srdata.nist.gov/cccbdb/).

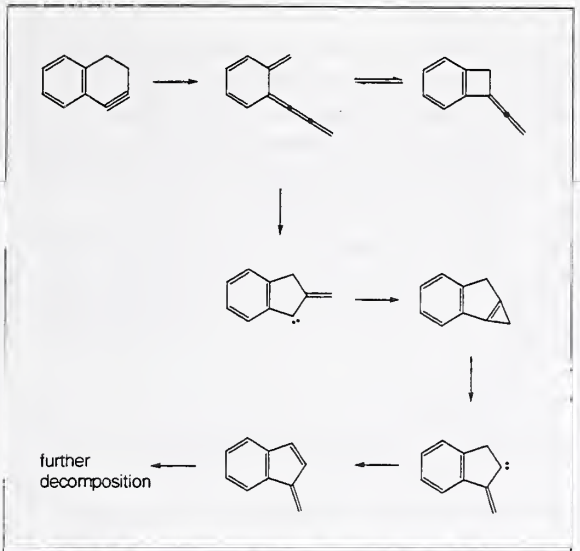
3. *Automated Predictions of Chemical Reactions and Their Mechanisms*

***K.K. Irikura** and R.D. Johnson III*

Objective: To predict the reactivity of molecules.

Problem: Chemical reactions are of essential and fundamental importance throughout chemistry and related technologies. Although experienced chemists can sometimes predict the reactions that will occur in a new chemical system, they may overlook some alternatives. Moreover, they are usually unable to make reliable predictions when the chemistry in question is unfamiliar to them. As more exotic chemicals and materials are investigated, this situation is increasingly common. Yet there are few tools available to assist in predicting chemical reactions, and none at all for predicting the novel reactions that are of greatest interest.

Approach: Quantum chemical calculations can predict how the energy of a chemical system changes as its constituent atoms move. This energy function, known as the potential energy surface (PES), contains all the information about the chemical reactions that are thermally possible in that system. Searching the PES will provide predictions of all those reactions. However, a typical PES has a high dimensionality, making it too large to search exhaustively. Thus, in practice it is impossi-



The starting compound was prepared in the hope that it would undergo the phenylcarbene rearrangement. However, isotopic labeling experiments, combined with the experience of the experimenters, indicated the mechanism shown above. It was recovered computationally using isopotential searching.

ble to find all possible chemical reactions. Nonetheless, by restricting the search to a contour of constant energy (isopotential), it is possible to find at least some reactions. Thus, this technique is a useful tool to supplement the predictions of a human expert.

Results and Future Plans: Several algorithms have been designed to implement the general task of isopotential searching, including one that is well suited for large-scale parallelization. Prototype software has been written and used to test the technique. The first tests were for chemical reactions that were predicted incorrectly by experts, with the correct results discovered later. In all cases, the correct reaction mechanisms were successfully produced by isopotential searching methods. A complex example is shown in the figure. In the future, we will (1) test the procedures on other reactions that are already known but that represent different types of chemistry (*e.g.*, transition metals), (2) apply the methods to make new predictions for important systems, and (3) distribute the software as appropriate.

Publication:

Irikura, K.K. and Johnson, R.D., III, ***"Predicting Unexpected Chemical Reactions by Isopotential Searching,"*** J. Phys. Chem. A (in press).

4. *Development of a General Purpose Geometry Optimizer for Large-Scale Molecular Systems*

C. Gonzalez and T. Allison

Objective: To develop and implement efficient geometry optimization algorithms aimed at the characterization of potential energy surfaces of large-scale molecular systems.

Problem: Recent advances in the efficiency of computational methodologies used in the evaluation of molecular energies and derivatives have created a need for efficient geometry optimization techniques. This need is particularly evident as the computational chemistry community attacks problems of increasing size and complexity such as biomolecules and reactions in condensed phases. While some of these techniques have appeared recently in the literature, they are typically integrated into specific codes and are not portable to other packages, restricting their access to a limited population of the scientific community. In addition, careful evaluation of the current geometry optimization packages has pointed to a marked lack of "user-friendly" tools that can aid in the semi-automated search for stationary points on potential energy surfaces of large molecules. Most of the time scientists need skills comparable to those of an expert in geometry optimization to tackle these problems. Given the increasing popularity of computational chemistry software in the study of a large variety of chemical problems, it is therefore necessary to build the appropriate infrastructure that will allow scientists to characterize the corresponding potential energy surfaces with a minimum of effort.

Approach: To address these needs, a general-purpose program with efficient optimization algorithms tailored for large molecules has been created. The program, called TURBO-OPT, performs geometry optimizations using energy derivative information from a variety of computational chemistry codes through a simple interface that gathers the necessary information from the normal outputs generated by these programs. This feature allows scientists to perform geometry optimizations using a common software platform that interfaces to the different theoretical methodologies available in common quantum chemical and molecular mechanics programs. For the advanced user, TURBO-OPT offers the necessary machinery to test and validate the different geometry optimization algorithms available in the literature.

Results and Future Plans: The current implementation of TURBO-OPT locates global and local minima, transition states, and reaction mechanisms. So far, the interface allows the use of two popular quantum chemistry codes. Preliminary tests, conducted on a series of 32 molecules with different degrees of freedom (5 – 500), show that the geometry optimization algorithms contained in TURBO-OPT provide substantial computational savings relative to conventional algorithms available in the literature. Efficiency in the algorithms is being improved and new features are being implemented. In addition, the interface will be extended so the program can be used with other computational chemistry packages. An alpha version of TURBO-OPT will be distributed among the members of the Computational Chemistry Group as well as selected scientists within NIST for validation purposes. It is expected that enough feedback will be gathered from the testing phase so that the current features of the program can be improved and extended to satisfy the demands of novice and advanced users.

5. *Measurements, Modeling, and Database Development for the Application of Alternative Solvents*

***T.J. Bruno** and A.F. Lagalante*

Objective: To develop and test predictive models for solvation of compounds in alternative solvents at supercritical, near-critical, and subcritical conditions using a combination of both physical and chemical variables as input into an empirical multivariate statistical model.

Problem: The most important thermophysical parameter required to assess the feasibility of an extraction process is the solute-solvent phase equilibrium. Serious limitations exist in equation-of-state approaches that use only physical properties of the solute and solvent to model phase equilibrium.

Approach: Safe replacements for conventional solvents are likely to come from fully or partially fluorinated alkanes, ethers, or ketones that possess negligible ozone depletion potential, as well as functionalized glycol ethers and siloxanes. Many of the fluorinated alternative solvents are gases under ambient conditions, and their thermophysical properties offer the promise of both conventional liquid extraction and the tunable solvent strength offered by near-critical and supercritical fluid extraction. In our approach, a given solution process is empirically modeled as the dependent variable in a multivariate statistical analysis. The independent variables to the multivariate statistical model include empirical solute-solvent interactions and additional state-dependent terms. Solute-solvent interactions are quantified using empirical solvatochromic and chromatographic parameters of acidity, basicity, polarizability, and polarity. These parameters represent the dominant chemical interactions in solvent-solute systems and will account for contributions to the nonideal portion of phase equilibrium. Accounting for these interactions will permit higher accuracy than EOS approaches. The statistical model aids in the identification of alternative solvents by making it possible to predict the solubility of industrially relevant compounds.

Results and Future Plans: In recent years, we have designed and constructed numerous spectroscopic, chromatographic, and gravimetric instruments for the measurement of solubilities of solutes in sub- and supercritical fluids. Solutes studied have ranged from classes of organometallic compounds to physiologically active natural products. The solvatochromic parameters for the fluorinated ethane solvents have been measured using high-pressure spectroscopic cells. Values are density-dependent over the gas-to-liquid density range and have been used to model R143a/water, R134a/water, and carbon dioxide/water partitioning of organic solutes. Parameters for the glycol ethers, alkanolamines, and siloxanes have been measured for both the pure compounds and aqueous solutions. Soon, we will be developing a fiber-optic solvatochromic sensor to facilitate measurement of solvatochromic parameters. The sensor will allow the rapid measurement of solvent mixtures for determination of mixture parameters. Pure component and mixture parameters will be incorporated into a database that will allow researchers to statistically model an industrially pertinent solvent replacement technology. The database model will suggest suitable alternative solvents and extraction conditions to substitute for a hazardous solvent.

Publications:

Lagalante, A.F. and Bruno, T.J., ***"Modeling the Water-R143a Partition Coefficients of Organic Solutes Using a Linear Solvation Energy Relationship,"*** J. Phys. Chem. 103, 7319 (1999).

Lagalante, A.F., Hall, R.L. and Bruno, T.J., ***"Kamlet-Taft Solvatochromic Parameters for the Fluorinated Ethane Solvents,"*** J. Phys. Chem. B 102, 660 (1998).

Lagalante, A.F., Wood, C., Clarke, A.M. and Bruno, T.J., ***"The Kamlet-Taft Solvatochromatic Parameters for 25 Glycol Ether Solvents and Glycol Ether Aqueous Solutions,"*** J. Sol. Chem. 27, 887 (1998).

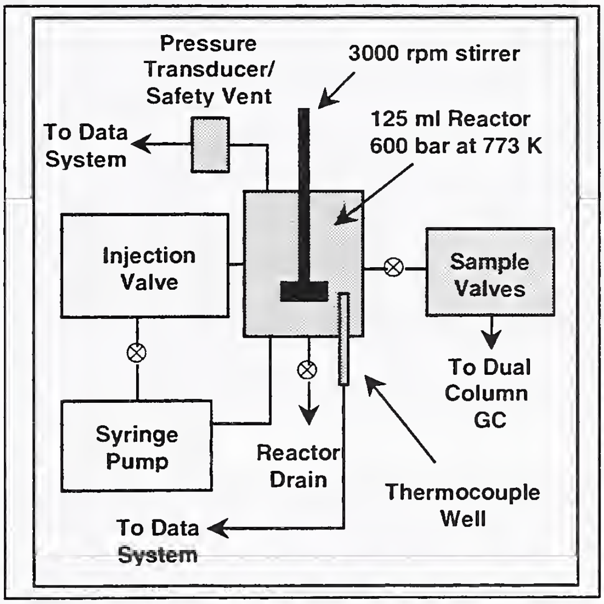
6. *Chemical Kinetics in Supercritical Water*

***J.A. Manion**, V. Anikeev, R.E. Huie, and W. Tsang*

Objective: To develop an experimental apparatus to obtain transferrable information on the rates and mechanisms of a variety of chemical reactions in supercritical water (SCW).

Problem: The physical properties of water change rapidly near its critical point (374 °C, 221 bar). One result is that rates and mechanisms of chemical reactions can be strongly affected by small changes in process variables. This makes the supercritical environment extremely interesting for use in hazardous waste destruction, the oxidation of biomass and metabolic wastes, and the development of new chemical synthesis strategies. A detailed understanding of the chemical reactions of organic compounds in SCW remains lacking, however, in part due to significant experimental difficult. For instance, the limited solubility of most organics in sub-critical water causes mixing difficulties and can necessitate complex corrections for diffusion. A more serious problem is that many reactions are faster in hot, dense water than in the supercritical environment. Classic static and flow systems involve long heat-up and cool-down times during which the organic is in contact with hot water. This can lead to spurious or ambiguous results. Finally, a widely applicable analytical system is needed, suited to studies of reactions about which little is known. Traditional post-reaction analysis, however, is both time-consuming and difficult in SCW studies because of phase separation of gases and of polar and non-polar liquids.

Approach. We have designed and built an innovative reactor that overcomes many experimental deficiencies of previous approaches. A schematic of the reactor is shown at right. The 125 mL static reactor is rated to 773 K and 600 bar, sufficient for studies with water densities ranging from gas-like to those approaching liquid water. The reactor features precise temperature and pressure controls and a stirrer rated to 3000 rpm. Water is added into the reactor using a high-pressure syringe pump. To avoid studying "hot water" chemistry, the organic of interest is injected directly into the pre-existing SCW environment. An automated high-pressure valve and loop system allows withdrawal and storage of micro-scale samples (6 μL) without perturbing the reactor conditions. Separate analyses of light gases and heavier components are performed on the dual-column GC. Sequential analyses allow the time progression of the reaction to be easily followed in a single experiment, vastly improving the efficiency of data collection.



Results and Future Plans. Systematic studies of the reversible dehydration of alcohols are underway. Results to date show that the rate of reaction is much faster in SCW than in the gas phase and is highly dependent on the density of the fluid. These observations suggest that ionic processes are important and occur even at relatively low fluid densities (0.2 g/cm^3). It is interesting to note that the reaction in SCW is significantly *slower* than in hot water. This demonstrates the importance of direct sample injection to obtain the correct kinetic parameters. Studies are being extended to other reaction classes involving hydrolysis and molecular reactions. In conjunction with the experimental program, we are collaborating with NIST molecular modelers and computational chemists to develop better theoretical models of reactions in SCW.

7. *Liquid, Vapor, and Gas Transport Properties in Membranes and Films*

J. Pellegrino, X. Yi, J. Portnoy, K. Nerbonne, and T. Rasco; O. Stange (GKSS Research Center); and M. Guiver (National Research Council of Canada)

Objective: To develop improved measurement methods for obtaining diffusion and solubility of liquids, vapors, and gases in membranes and films, to elucidate transport mechanisms and quantitative structure/transport property prediction methods for membrane materials (especially polymeric) based on high quality measurements of sorption and transport in several well-characterized systems, and to compile property data on industrially important materials used for membrane-based separations.

Problem: Although polymeric and inorganic materials are used in membrane and adsorptive separation processes, a significant barrier to the optimum use of existing materials and development of new materials is the lack of predictive capabilities for the transport properties of mixtures in any selected material. Improved processes for obtaining high-purity oxygen and nitrogen from air, processing natural gas, recovering hydrogen from refinery streams, recovering and purifying olefin streams, and purifying water are examples of important industrial uses of membranes.

Approach: This program has measurement, modeling, and database components. Measurements of liquid, vapor, and gas diffusion and sorption in thin layer films are critical for development of techniques to predict membrane transport properties. These measurements provide a means to include the effects of both chemical and structural subgroups in the material, and ultimately, to delineate rational design criteria for separations. Through our collaborations we have access to materials for which chemistries are well studied and/or can be varied in well- defined ways. In addition, the polymers currently under study (polypropylene, cellulose acetate, polysulfones, polyperfluorosulfonic acid, polytrimethylsilyl-propyne, and polyaniline) represent both commercial and newly developed materials. This research program also includes the development of an internet-accessible database of polymeric material properties important for membrane separation design.

Results and Future Plans: A flow cell equipped with attenuated total reflectance (ATR)-FTIR and an accurate flow control and measurement system has been developed to measure diffusion of multi-component mixtures in films. Two techniques for making measurements on pre-made films (necessary for making measurements on commercial membranes) have been developed. Using one of the techniques, measurements of water and acetone mixtures diffusing from the liquid state into a commercial polypropylene (PP) film have shown that (1) water must lose its H-bonding before entering PP and (2) in a mixture, acetone diffusion is coupled with water and speeds up the process. The second technique, using a thin (<0.5 μm) adhesive layer (*e.g.,* of a mineral or fluorochemical oil) to maintain good optical contact between a pre-made film and the ATR crystal, will facilitate measurements with gas mixtures. The sorption program includes four sorption apparatus (based on pressure decay methods) that, during the past year, were used to determine the film density of polyvinyl alcohol-modified with cyclodextrin side groups. A surface acoustic wave device will be brought into service during the coming year with the ultimate objective of combining it with the ATR-FTIR flow cell for multicomponent gas and vapor transport measurements. An initial version of the membrane technology database, containing unevaluated gas transport properties on hundreds of polymers, has been completed and is accessible via the internet.* This work begins to address a need within the chemical engineering community for comprehensive, critically evaluated information on separation membranes, and how these membranes interact with important chemical feedstock components. The database includes permeability, solubility, and diffusion coefficients; ideal and mixed gas separation factors; temperatures; primary reference; monomer repeat unit structure; and common names and abbreviations. Future work will expand the number of polymers and include predictive models.

*(http://www.membranes.nist.gov).

8. *Measurements and Data for Pressure-Driven Membrane Separations*

J. Pellegrino, E.J. Han, and M. Lewis; G. Amy, J. Cho, Y. Yoon, P. Brandhuber, S. Wright, and S. Delagah (Univ. of Colorado); and M. Chapman Wilbert and K. Price (U.S. Bureau of Reclamation)

Objective: To develop improved quantitative characterization techniques and predictive models for the filtration of complex mixtures using commercial membranes based on high quality measurements of streaming potential and water transport coefficients and detailed measurements of filtration results on dilute, complex aqueous mixtures encountered in membrane-based separations.

Problem: The first commercially viable, synthetic membranes suitable for molecular scale separations (reverse osmosis, nanofiltration, and ultrafiltration) were developed over twenty-five years ago. However, research during the intervening years has not developed a systematic approach for matching membranes to complex mixtures and predicting the filtration figures-of-merit: species partitioning into the membrane (rejection), solvent (water) permeability, and permeability decline with time. Improved processes for obtaining specialty chemicals, pharmaceuticals, and advanced monomers using environmentally benign processes, and more economic ways to recover, reuse, and supply water are examples of important industrial and municipal uses of membranes.

Approach: This program has both measurement and modeling components. Meaningful and accurate measurements on both the membrane and the complex mixtures are required in order to develop a systematic correlative approach. These measurements provide a means to combine the effects of chemical, physical, and structural characteristics of the membrane and the mixture, and ultimately, to delineate rational design criteria for separations. Through our collaborations with the U.S. Bureau of Reclamation and the University of Colorado, we are developing new test protocols, refining existing characterization techniques, and developing a database of consistent measurements of filtration figures-of-merit and membrane and mixture characteristics. This database is being compiled to facilitate the development of correlative models for matching membranes to specific applications.

Results and Future Plans: This year we have used our improved protocol for measuring the tangential flow streaming potential of membrane sheets. This technique is commonly used to characterize the relative surface energy and charge at the membrane interface. We are measuring streaming potential as a function of electrolyte composition, concentration, pH, and temperature. These data will be used in a model to calculate the surface potential of the membrane or film. The membrane's surface potential will then be incorporated into materials research, manufacturing quality control, and engineering design models. We have continued development and testing of a new apparatus to measure the kinetics of solvent diffusion through membranes. This apparatus has a resolution on the order of 10^{-8} L/s and may provide an improved method for absolute characterization and monitoring of very subtle structural changes in membrane materials, caused by aging, exposure to chemicals, and mechanical trauma. We were able to successfully use this apparatus to identify small structural changes in reverse osmosis membranes exposed to dilute NaCl solutions versus control samples. We have extended our filtration database measurements beyond natural organic matter filtration to include trace hazardous species, for example, arsenate, arsenite, and perchlorate ions, and colloidal particles. We have also developed a semi-empirical model for modeling and predicting flux decline in macromolecule filtration that is mostly based on parameters that may be measured independently or estimated from physicochemical properties of the solutes.

Publication:

Chapman-Wilbert, M., Delagah, S., and Pellegrino, J., ***"Evaluation of Variance in Streaming Potential Measurements,"*** J. Membrane Sci. 161, 247 (1999).

9. *Structure, Adsorptive Separations, and Characterization of Surfactant/Clay Complexes*

C.D. Muzny, T.J. Bruno, and H.J.M. Hanley

Objective: To exploit the unique characteristics of clay platelets in the production of organic-inorganic composite materials with revolutionary material properties and in the development of novel chemical separation techniques.

Problem: Clay is the key inorganic substance in applications ranging from pollution prevention and remediation, enhanced oil recovery, the treatment of petroleum liquids, the manufacture of cosmetics and pharmaceuticals, and the synthesis of polymer nanocomposite materials. An understanding of clay-organic chemical interactions and the effects these interactions have on the structure of clay complexes is a key issue for future developments in all of these applications.

Approach: Our approach is twofold. First, in order to understand the structural changes induced by clay surface treatments, we are undertaking a small-angle neutron scattering and dynamic light scattering investigation of the complexes formed in mixture suspensions of clay mineral and cationic surfactants. These techniques allow us to monitor the changes in the nanoscale structural properties of clay and organoclay complexes in a variety of situations. Second, in order to understand the chemical kinetics of the clay-organic interaction, we have advanced the application of physicochemical gas chromatography by devising stable clay- and organoclay-coated capillary columns. The capillary column approach that we have developed is more efficient, requires lower column temperatures, and produces values of the enthalpy of adsorption (H_{ads}) with a much lower uncertainty than the conventional techniques.

Results and Future Plans: Our results are wide ranged. For example, they include elucidation of the surface structure of synthetic clay Laponite with cetyltrimethylammonium bromide (CTAB) complexes; determination of the effect of a mineral surface on micelle formation; investigation of the effect of shear on colloidal gel formation using an adapted Couette cell of a constant stress rheometer which is placed in the neutron or light beam; investigation of the formation and structure of complexes of organic macromolecule adsorbed on an inorganic substrate; and investigation of dispersion and flocculation in aqueous mineral systems. We have also determined H_{ads} for a family of hydrocarbons on Laponite and Laponite complexed with CTAB by application of the organoclay-coated capillary column gas chromatography technique. The CTAB-coated Laponite is especially significant in the environmental context in that it represents a surrogate soil system and can be used to understand the interaction of pollutants on soils.

Future plans are to investigate further the relationship between the structure and rheology of gelling systems; to attempt to construct and characterize clay nanocomposites formed in an organic medium; and to understand better the role of an organic surface on flocculation mechanisms. We would also like to co-ordinate the structure surface studies with the thermodynamic information obtained from the coated capillary column. In addition, our future plans include extending the chromatographic technique to the measurement of the diffusion of pollutants into the clay and organoclay system.

Publications:

Bruno, T.J., Lewandowska, A., Tsvetkov, F., and Hanley, H.J.M., ***"Determination of Heats of Adsorption on a Synthetic Clay by Gas-Solid Chromatography Using a Wall Coated Open Tubular Column Approach,"*** J. Chromatogr. A 844, 191 (1999).

Hanley, H.J.M., Muzny, C.D., and Butler, B.D., ***"Surface Adsorption in a Surfactant/Clay Mineral Solution,"*** Int. J. Thermophys. 19, 1155 (1998)

10. *Thermophysical Properties of Gases Used in Semiconductor Processing*

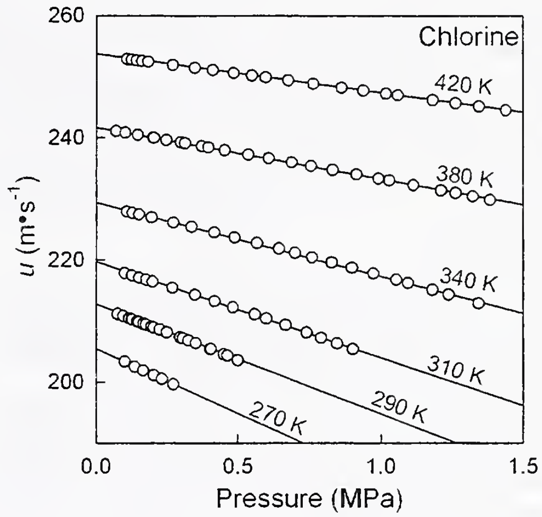
***J.J. Hurly**, K.A. Gillis, and M.R. Moldover*

Objective: To provide high-accuracy data for modeling chemical vapor deposition (CVD) and for calibration of mass flow controllers (MFCs) used in semiconductor processing.

Problem: Many process gases are toxic, corrosive, and/or pyrophoric. For such gases, measurements of thermophysical properties are sparse and rarely accurate. But accurate data are required to model the hydrodynamics of the gas streams, *i.e.*, the velocity and temperature profiles in the vicinity of the hot susceptor, and the hydrodynamics that evolve within the streams used in CVD processes. MFCs are used to deliver process gases (*e.g.*, Cl_2, HBr, BCl_3, WF_6) for CVD and for other processes (*e.g.*, plasma etching). Calibrated MFCs are needed to scale processes up from prototype to pilot plant and to production. Although MFCs are used with process gases, they are sold with calibrations for surrogate gases. Because the operation of MFCs depends upon heat transfer, converting the calibration from a surrogate gas to a process gas requires the heat capacity, thermal conductivity, density, and viscosity as functions of temperature and pressure.

Approach: We are using acoustic techniques to measure the thermophysical properties of three classes of gases: (1) binary mixtures of CVD carrier gases with process gases, (2) pure process gases, and (3) surrogate gases. We will develop a comprehensive, reliable database for these gases that provides the heat capacity, thermal conductivity, viscosity, and the pressure-density-temperature relation for the gases and also diffusion coefficients for mixtures of the gases. The diffusion coefficient will be obtained from models for the intermolecular potentials between the carrier and the process gases.

Results and Future Plans: We developed a facility for safely measuring the properties of these hard-to-handle gases. During the past year, we have completed measurements on the seven gases identified by the SEMATECH MFC Working Group as having the highest priority. The figure displays speed-of-sound data for chlorine. The data range from somewhat below the boiling temperature to 200 °C and from 25 kPa to 1500 kPa or 80% of the vapor pressure. The data were analyzed for the ideal-gas heat capacity and the equations of state with uncertainties of approximately ±0.1%. For all seven gases, effective pair potentials have been derived and these pair potentials have been used to estimate the transport properties of these gases. In the coming year, the speed-of-sound will be measured in an organometallic gas and in other process gases. Acoustic measurements of the transport properties and a database are planned.



Publications:

Hurly, J.J., *"Thermophysical Properties of Gaseous CF_4 and C_2F_6 from Speed-of-Sound Measurements,"* Int. J. Thermophys. 20, 455 (1999).

Hurly, J.J., Defibaugh, D.R., and Moldover, M.R., *"The Thermodynamic Properties of Sulfur Hexafluoride,"* Int. J. Thermophys. (in press).

Hurly, J.J., *"Thermophysical Properties of Gaseous Tungsten Hexafluoride from Speed-of-Sound Measurements,"* Int. J. Thermophys. (in press).

11. *Experimental and Theoretical Determinations of the Mechanisms, Kinetics, and Thermochemistry of Chlorinated Species*

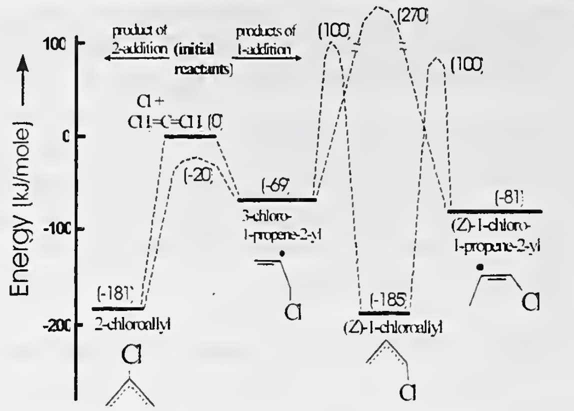
J.W. Hudgens, J.A. Manion, C. Gonzalez, K.K. Irikura, and T.C. Allison

Objective: To measure and predict the reaction mechanisms, kinetics, and thermochemistry of C_3 (and larger) chlorinated species.

Problem: Within incinerators and plasmas, the reactions of chlorine with unsaturated C_3 chemical species are believed to engage in sequences that synthesize highly chlorinated by-products and pollutants. Yet, when attempting to formulate a numeric model that describes the production of such chlorinated species, one finds that no reliable reaction mechanisms, rate coefficients, or thermochemical data are available. The absence of such data persists because many practical obstacles have hindered experimental measurements of these properties. Moreover, in the absence of benchmark experimental data, the *ab initio* computational community has generally ignored the entire chemical class of C_3 chlorinated species, and thus, no broad overview for these species exists.

Approach: The research acquires new experimental kinetic and thermochemical data and involves extensive, state-of-the-art *ab initio* calculations, enabling interpretation of the data. We attempt to formulate or adapt models that allow us to predict trends across the entire chemical class. Cavity ring-down (CRD) absorption spectroscopy is used to measure real-time kinetic data and gas-chromatography/mass spectrometry (GC-MS) is used to measure the reaction end-products. The *ab initio* methods use density functional, Møller-Plesset, and multi-reference codes and formulations involving isogyric and isodesmic reactions.

Results and Future Plans: Two successful studies have shown: (1) new insights into the reactions of chlorine atoms with unsaturated C_3 species and (2) the inability of older theories to predict the properties of perchlorinated compounds. In the first study, we used CRD and GC-MS experiments to measure the reaction rate coefficients and end-products of the reactions, Cl + allene and Cl + propargyl chloride. Both reactions form energized radicals that isomerize before forming products. By using *ab initio* calculations to predict each governing reaction surface (*e.g.*, figure), we could accurately predict the observed products and confirm the governing mechanisms. The work also lead to new thermochemical enthalpies for several chlorinated C_3 radicals and stable species.



Ab initio *energy diagram that explains the products observed from Cl + allene.*

The second study tested the accuracy of the widely-used "group additivity" tools for predicting the enthalpies of formation, $\Delta_f H^\circ_{298}$, for C_1, C_2, and C_3 chlorocarbons. This research compared experimental enthalpies with those predicted by extensive *ab initio* calculations and several group additivity methods. Of particular interest was the recently determined $\Delta_f H^\circ_{298}$ for perchloropropene which provided a rigorous test for these predictive methods. In short, this extensive work found that modified group additivity works well for C_2 species, but no group additivity method gives reliable values of $\Delta_f H^\circ_{298}$ for highly chlorinated C_3 species.

12. *Evaluated Data and New Computational Tools for Chemical Reaction Engineering*

D.R. Burgess

Objective: To develop computational tools that utilize thermochemical and chemical kinetic data for the modeling of chemical mechanisms, and to validate these tools through comparison with evaluated data.

Problem: Many computational tools used in chemical reaction engineering have limited ability to assign rigorous quantitative uncertainties to the results of the calculations. In addition, the evaluated data for benchmarking the computational models are often not readily available.

Approach: Data are evaluated for developing robust chemical kinetic measurements. This evaluation involves a synthesis of experimental data and computational predictions as a means of verifying the quality of the data. The primary goals are to provide high quality thermochemical functions and rate expressions. A secondary focus is to determine procedures for providing quantitative uncertainties to values that are traceable to *ab initio* calculations (energetics) and solutions to the master equation (rate expressions). We are also developing tools for managing the thermochemical and chemical kinetic data necessary for reacting flows simulation and for reaction path analysis and mechanism generation/reduction.

Results and Future Plans: Current systems of interest are hydrocarbon combustion and halogenated hydrocarbon destruction chemistries. We have compiled, calculated, and evaluated thermochemical data for the C_1 and C_2 fluorinated hydrocarbons. We have compiled experimental rates of reactions and calculated *ab initio* transition states for HF elimination pathways from the fluoromethanes and fluoroethanes. The geometries and energies of the transition states are determined from high-level *ab initio* quantum chemistry calculations employing the G2 and CBS methods. The transition states are then used as inputs to master equation calculations, which yield temperature and pressure dependent rate expressions. We have had significant success in validating the calculated rate expressions against experimental data and have identified previously unrecognized decomposition channels. We have now begun *ab initio* transition state calculations for thermal decomposition of the C_1 and C_2 chlorinated hydrocarbons.

In a related effort, we are working with Reaction Design Corporation in (1) developing database tools for managing the thermochemical and chemical kinetic data needed in reacting flows simulations; (2) implementing Deterministic Equivalent Modeling Method (DEMM), a recently developed computational tool, which provides quantitative uncertainties for simulation results if uncertainties are input for the thermochemical and chemical kinetic data; (3) implementing computational tools for reaction path analysis and mechanism generation/reduction (*e.g.*, Principal Component Analysis); and (4) assigning computationally useful uncertainties to recommended rate expressions for hydrocarbon combustion chemistries for use in DEMM-based calculations.

13. *Fundamentals of Fire Suppression Through Computer Simulations*

***W. Tsang**, V. Babushok, and D.R. Burgess*

Objective: To develop an understanding of fire suppression from a fundamental point of view and to make contributions to efforts to find replacements for presently used agents through the use of computer simulations.

Problem: The phase-out of traditional fire suppressants owing to the effect of these suppressants on the ozone layer has led to much interest in alternative compounds. The traditional method for discovering new suppressants is through empirical testing. Computer simulations represent a potentially new tool to expand and focus experimental efforts.

Approach: With the increasing availability of powerful computational tools, the prerequisite for accurate results from computer simulations is a reliable data base of the rate constants for the fundamental chemical interactions and the thermodynamic properties of the compounds responsible for the suppression process. These were determined from an evaluation of direct experimental measurements and through the use of various estimation methods. As much as possible, results were validated through comparisons with test results carried out in the Fire Research Group at NIST. Various possible markers for suppressant effectiveness were examined. Fits with experimental results were optimized. The optimized model was then used to answer a number of general and long-standing questions on the nature of fire suppression.

Results: The decrease in laminar flame velocity as a function of additive concentration is used as a measure of suppressant efficiency. Simulation studies and a detailed examination of the chemistry confirmed that the changes are a consequence of the reduction of the active flame radicals such as H, OH and O and that the existence of catalytic cycles controls the effectiveness of a suppressant. Thus although fluorine can remove hydrogen atoms, the hydrogen fluoride that is formed cannot be recycled. In contrast, for a bromine compound, in the sequence of reactions $H+HBr=H_2+Br$ and $Br+RH=HBr+R^*$, HBr is acting as a catalyst. The existence of a reliable model is especially valuable in answering broad questions and for setting limits and directions of future work. The relative importance of chemical and physical effects on fire suppression can be easily settled by simply "turning off" the chemistry. For CF_3Br, the chemical component is responsible for about 80% of the initial decrease in the flame velocity. Inversely, one can "turn on" the chemistry. We found that under such conditions, one must have concentrations in the high tens or low hundreds ppm. Such a criterion is in fact met by iron compounds. A consequence of this is that the mechanism for inhibition for such compounds cannot involve gas-solid reactions. Condensation will lead to a further decrease in suppressant concentration, and rate constants are already at a maximum. Indeed, the modeling shows that the condensation of iron compounds leads to a decrease in suppressant efficiency. Another interesting issue is the applicability of experimental and modeling data carried out with a particular fuel to other fuels. Through sensitivity analysis with a variety of fuels, we demonstrate that in practically all cases the decrease in the flame velocity was controlled by the same set of reactions. This finding justifies the use of a universal ranking of suppressant activity.

14. *Photochemical Reduction of CO_2 Catalyzed by Metal Complexes*

P. Neta and J. Grodkowski (Guest Researcher)

Objective: To obtain kinetic and mechanistic information on the elementary reactions involved in the photochemical reduction of CO_2 and to develop strategies for conversion of CO_2 into a fuel or feedstock materials.

Problem: Accumulation of CO_2 in the atmosphere from the burning of fossil fuels leads to global warming. It would be advantageous to reduce the amount of CO_2 by converting it into useful chemicals. Reduction of CO_2 can form various compounds including, CO, HCOOH, CH_2O, CH_3OH, and CH_4.

Approach: Iron and cobalt porphyrins and related compounds are studied as catalysts for photochemical reduction of CO_2. Our approach is to attempt to use them in photochemical systems, to demonstrate formation of CO and/or HCOOH from CO_2, and then to examine the mechanism of catalysis and to determine the relevant rate constants by pulse radiolysis.

Results and Future Plans: We have found that iron and cobalt metalloporphyrins (MP) act as effective catalysts for the photochemical reduction of CO_2 to CO and formic acid in dimethylformamide or acetonitrile solutions containing triethylamine as a reductive quencher. In these solutions, $M^{III}P$ is reduced ultimately to M^0P, which reacts with CO_2 to form CO. In these photochemical studies, the quantum yields were low. In a recent study we have shown that the yield can be dramatically increased by the use of *p*-terphenyl (TP) as a photosensitizer. TP is very effectively photoreduced by triethylamine (TEA) to form the radical anion, $TP^{\bullet-}$, which can reduce Co and Fe porphyrins rapidly to the M^0P state. The metalloporphyrins were destroyed during the photochemical process and yet production of CO continued. These findings suggest that catalytic reduction of CO_2 to CO may be affected by the ferrous ions formed after decomposition of the porphyrin ligand. We have found that the mechanism involves different intermediates. The $TP^{\bullet-}$ radical anion reduces Fe(II), and the Fe(I) ions produced react with CO_2 to form an adduct. Subsequent reduction of the Fe-CO_2 adduct by $TP^{\bullet-}$

or by Fe(I) leads to formation of CO. After extensive irradiation, photochemical production of CO stops. This is caused by competition between CO and CO_2 for the Fe(I) binding sites. In all of the above experiments, the catalysts are either destroyed by side reactions or deactivated by attachment of CO. We plan to investigate various strategies to overcome these limitations. A promising route may be the incorporation of the catalysts into solid support, which will protect against undesired side reactions and/or permit recovery and reuse of the catalysts.

Publications:

Dhanasekaran, T., Grodkowski, J., Neta, P., Hambright, P., and Fujita, E. ***"p-Terphenyl Sensitized Photoreduction of CO_2 with Cobalt- and Iron-Porphyrins. Interaction Between CO and Reduced Metalloporphyrins,"*** J. Phys. Chem. A 103, 7742 (1999).

Neta, P., ***"Radiation Chemical Studies of Porphyrins and Metalloporphyrins,"*** in Radiation Chemistry: Present Status and Future Prospects, C. D. Jonah and B. S. M. Rao, eds., Elsevier, 1999 (in press).

Grodkowski, J. and Neta, P., ***"Cobalt-Corrin Catalyzed Photoreduction of CO_2,"*** J. Phys. Chem. (in press).

Grodkowski, J. and Neta, P., ***"Ferrous Ions as Catalysts for Photochemical Reduction of CO_2 in Homogeneous Solutions,"*** J. Phys. Chem. (in press).

15. *International Standards for Refrigerant Properties*

M.O. McLinden, A. Laesecke, E.W. Lemmon, and R.A. Perkins

Objective: To facilitate and promote international standards for the thermodynamic and transport properties of refrigerants.

Problem: The hydrofluorocarbons (HFCs) and other fluids are now seeing widespread commercial use in place of the ozone-depleting CFC and HCFC refrigerants. To evaluate the energy efficiency, capacity, *etc.*, of any fluid in a thermodynamic cycle, knowledge of the thermophysical properties is required. Standards exist for determining and reporting the performance of air-conditioning systems at standard ratings conditions. But differences between multiple, "competing" property formulations lead to differing performance ratings. This is especially a problem in international trade.

Approach: We work with several international groups that develop and/or promulgate standards. Chief among these is Annex 18 of the International Energy Agency, a group that NIST organized in 1990. We are also active in the Transport Properties Subcommittee of IUPAC. Finally, the NIST REFPROP database has, itself, been adopted as a *de facto* standard within the refrigeration industry. We work within the IEA and IUPAC groups to promote REFPROP as the source of refrigerant property data. As new standards are adopted, we revise REFPROP, if needed, to conform to the new standards.

Results and Future Plans: The IEA Annex 18, Thermophysical Properties of the Environmentally Acceptable Refrigerants, concluded its third and final phase in 1999. The Annex carried out comprehensive evaluations of the available equations of state and sanctioned standards for R123, R134a, R32, R125, and R143a. Wide participation was invited in this process, and anyone could submit an equation of state for evaluation. Of the five fluids, the formulations for R123 and R143a developed at NIST were designated as international standards. A similar comparison of mixture models has facilitated the dissemination and adoption of a new mixture modeling approach. This model, based on Helmholtz energies for each of the mixture components and developed at the University of Idaho and NIST, is implemented in REFPROP and also forms the basis for an extensive tabulation of properties prepared by the Japan Society of Refrigerating and Air Conditioning Engineers. The final report for Annex 18 was presented at the quadrennial Congress of the International Institute of Refrigeration.

Under the auspices of the IUPAC Subcommittee on Transport Properties, NIST acts as one of three coordinators of a project on "Thermochemical, Thermodynamic and Transport Properties of Halogenated Organic Compounds and Mixtures." In the past NIST has organized, through IUPAC, an international round robin comparison of the viscosity and thermal conductivity of R134a. We are now working to develop formulations for propane, butane, and isobutane (so-called "natural refrigerants") that are of increasing interest.

The ISO has recently approved the establishment of a working group to establish standards for refrigerant properties. NIST will be active in this group, along with many of the Annex participants.

Publications:

McLinden, M.O. and Watanabe, K., ***"International Collaboration on the Thermophysical Properties of Alternative Refrigerants: Results of IEA Annex 18,"*** Proc. 20th Int. Congress of Refrig., Sydney, Australia, September 19-24, 1999, Int. Inst. Refrig.

Lemmon, E.W. and Jacobsen, R.T., ***"An International Standard Formulation for the Thermodynamic Properties of 1,1,1-Trifluoroethane (HFC-143a) for Temperatures from 161 to 500 K and Pressures to 60 MPa,"*** J. Phys. Chem. Ref. Data (in press)

16. *Thermophysical Properties of Natural Gas Systems*

R.A. Perkins, *E.W. Lemmon, T.J. Bruno, D.G. Friend, A.H. Harvey, C.D. Holcomb, M.L. Huber, A. Laesecke, J.W. Magee, M.O. McLinden, S.L. Outcalt, J.C. Rainwater, J.L. Scott, W.M. Haynes, I.M. Abdulagatov (Dagestan Scientific Center), and S. Kiselev (Inst. Oil and Gas Res., Russia)*

Objective: To measure accurately the thermophysical properties of natural gas mixtures and develop standard reference models that are internationally accepted for calculating properties within the required uncertainties of the data over large ranges of temperature, pressure, and composition.

Problem: The thermophysical properties of natural gas systems must be accurately known for national and international custody transfer. It is not possible to measure all possible compositions of natural gas; thus, accurate predictive models are required by industry. These models must be validated with reliable data obtained on a limited number of samples that have well defined compositions. The nature of custody transfer in gas pipelines and liquefied natural gas shipping requires that these models be recognized as national and international standards. Custody transfer also requires that the gas satisfies certain quality (low concentrations of hydrogen sulfide) and odorant safety standards.

Approach: The natural gas systems selected for experimental study are determined by comparisons of the best available models with existing data for systems that are of interest to industry. These comparisons identify systems where additional data are required to fill significant data gaps or where unresolved discrepancies exist between several data sets. Improved Helmholtz energy formulations, which also allow calculation of all thermodynamic properties in the fluid phases of a mixture system in a consistent manner, offer potential for reduced uncertainty for a wider range of mixture systems. Experimental data obtained at NIST on gravimetrically prepared mixtures will extend and enhance the data available in the literature to develop accurate mixture models and to validate the performance of new mixture models. NIST has also been measuring the diffusion coefficient of odorant compounds in gas mixtures in an effort to understand the problem of odorant fading. NIST is currently making measurements of the kinetics and catalysis of the hydrolysis reaction of carbonyl sulfide in propane. This hydrolysis can generate unacceptable levels of hydrogen sulfide in natural gas during transmission.

Results and Future Plans: The Gas Processors Association funded a five-year project to study high pressure gas separation and conditioning which will include phase equilibrium, co-existing density, surface tension, and viscosity measurements and model development. PVT measurements were completed on three mixtures of CO_2 + ethane at temperatures from 200 K to 400 K with pressures to 35 MPa. A paper describing the PVT and isochoric heat capacity measurements on two mixtures of propane and isobutane is in press. Measurements have been completed on the thermal conductivity of propane at temperatures from 83 K to 600 K with pressures to 70 MPa. Although the data are in very good agreement with several reliable researchers, deviations between the best available model and these data reach 10 % at high temperatures. Measurements have been completed on the viscosity of propane and isobutane at temperatures from 300 K to 420 K with pressures to 70 MPa. An improved correlation was published in the Journal of Chemical and Engineering Data in collaboration with IUPAC on the viscosity of propane. These measurements enable improved corresponding states predictions (propane reference fluid) of natural gas mixture viscosity and thermal conductivity. Measurements are in progress on the thermal conductivity of isobutane and the viscosity of normal butane. A mixture model, based on a generalized corresponding-states algorithm for the excess Helmholtz energy and reference quality formulations for the constituents, has been developed. Long-term plans involve the addition of other fluids such as the heavier hydrocarbons, helium, hydrogen, water, carbon monoxide, and hydrogen sulfide. Modeling work this year has focused on addition of helium, hydrogen, water, and pentane and higher hydrocarbons. NIST is also evaluating the catalytic effects of wetted materials such as stainless steels and aluminum alloys on the kinetics of the hydrolysis reaction of COS in propane.

17. *Properties for Advanced Hydrogen Technologies*

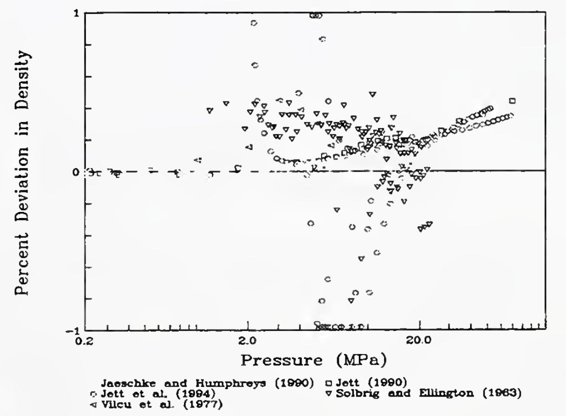
***D.G. Friend**, M.L. Huber, E.W. Lemmon, G.R. Hardin, and J.C. Rainwater*

Objective: To provide industry with high quality thermophysical property surfaces for mixtures of hydrogen and methane over broad ranges of temperature, pressure, and composition.

Problem: There are currently no high accuracy models available that can handle mixtures of hydrogen and methane at high hydrogen concentrations, although fuel cells and hydrogen technologies may play a more important part in satisfying our energy needs. The fuel processing stage in fuel cells, known as reforming, involves processing the fuel to separate hydrogen from the other constituents, and mixtures of hydrogen and methane may be found in this sub-system of a fuel cell. Mixtures of hydrogen and methane have also been proposed as a fuel that may be used directly in internal combustion engines to reduce CO_2 and NOX emissions. The proposed research will develop a model for predicting the thermophysical properties of hydrogen/methane mixtures over the entire composition range from pure hydrogen to pure methane. The topic of this report relates to a project funded by the Electronics and Photonics Technology Office of the ATP program and is part of a more extensive program on fuels and, in particular, on fluids related to natural gas systems. When the cryogenic fluids, hydrogen and helium, are included in such fluids, the standard property formulations must be reconsidered in part because the typical phase envelope topology is 1^C1^Z rather than 1^C as for most other component pairs in natural gas systems.

Approach: Two models which are currently being used to establish standard reference thermodynamic surfaces are the extended corresponding states (ECS) model and a two-fluid Helmholtz mixing model. Both of these can use existing high accuracy pure fluid equations of state for methane and hydrogen, so that the mixture model will reduce to the pure fluid standards in the proper limits. The first step in the project is to perform a literature search and collect and evaluate experimental thermodynamic data (PVT relationships, heat capacities, vapor-liquid equilibria, sound speeds) for the methane/hydrogen binary system. Versions of both the ECS and Helmholtz mixing models were developed to describe the data, and the behavior of the binary interaction parameters was investigated. As the models are developed, comparisons will be made with the experimental database. Upon achieving a satisfactory optimized model, it will be incorporated into a NIST Standard Reference Data mixture database.

Results and Future Plans: There are about 3000 experimental points from 25 sources which give relevant thermodynamic data for the hydrogen-methane system. Although the data situation for the mixture is generally satisfactory, data are sparse for concentrations near the equimolar composition and at the lower temperatures; in addition, there are no caloric data which are generally required to establish the most accurate property standards. Initial results for the ECS model exhibited some numerical convergence problems; thus, much of the development and optimization work has been completed on the two-fluid Helmholtz energy model. Sample



deviations between the data and model are shown in the figure. These results have been implemented in a version of NIST Standard Reference Database 14, although further testing, optimization, and quality control protocols will be required before releasing the revised database.

Future work will concentrate on additional mixtures of natural gas components with hydrogen, *i.e.*, mixtures with ethane, propane, higher alkanes, and multiple components. Although some of the current generation of test vehicles operating on hydrogen-enriched fuels use hydrogen-methane mixtures, the more general fuel will be a mixture of hydrogen and natural gas from an arbitrary source. Results will be incorporated into the NIST property infrastructure as represented by the PC and web-based databases.

18. *Transport Properties of Refrigerants and Refrigerant Mixtures*

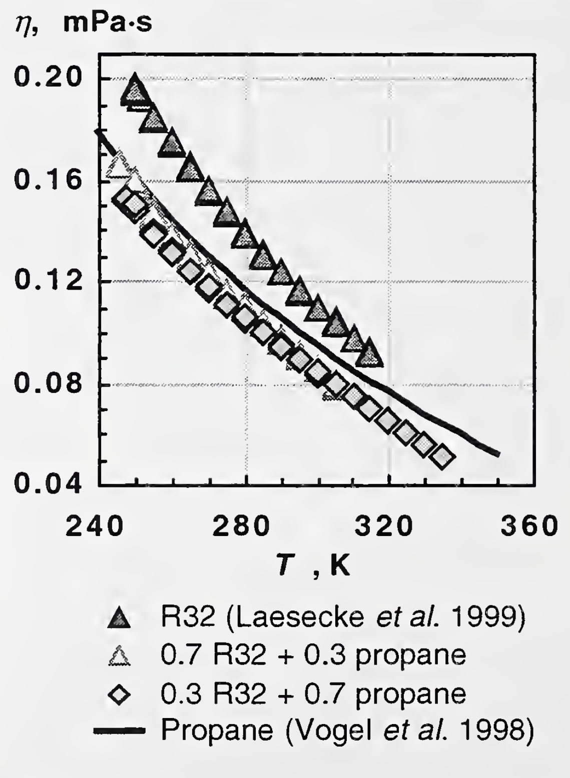
A. Laesecke, R.A. Perkins, M.O. McLinden, and M.L. Huber

Objectives: To resolve large discrepancies between literature data for the transport properties of pure refrigerants and to provide reliable experimental transport properties data for the refrigerant mixtures to develop advanced property models.

Problem: Viscosity measurements for alternative refrigerants, which were carried out in different laboratories since 1988, exhibited differences up to 35%, far in excess of experimental uncertainty. Lack of experimental transport properties data for alternative refrigerant mixtures impedes model refinement and the use of such mixtures in HVAC equipment.

Approach: The NIST sealed gravitational viscometer with a straight vertical capillary was used for new benchmark measurements of saturated liquid ammonia, R32, and R134a to resolve the disconcerting discrepancies between literature viscosity data. Viscosity and thermal conductivity measurements were carried out on four binary and one ternary blend of R32, R125, R134a, and propane (R290), each at two compositions. Measured conditions included subcritical liquid and vapor as well as supercritical phases. Viscosities were measured in the sealed capillary viscometer and in the torsional crystal viscometer. Thermal conductivities were determined from transient and steady-state measurements in hot-wire instruments. These data are used to develop improved transport property models.

Results and Future Plans: Some of the literature data sets did not properly apply necessary corrections in their analysis. Agreement within the combined experimental uncertainty was achieved after applying these corrections. An improved correction for the radial acceleration in viscometers with coiled capillaries was developed. A need was identified to extend international viscometry standards to sealed gravitational capillary instruments. Present standards cover only open capillary viscometers which cannot be used for measurements of volatile liquids. Transfer of sealed viscometer technology to a manufacturer is underway. The mixture measurements revealed strongly non-ideal composition dependences for transport properties in systems of nonpolar/polar compounds such as R32 + propane. The figure shows that the saturated liquid viscosities of both blends are even lower than the viscosity of propane. These results will be incorporated in improved mixture transport property models. The measurements with the torsional crystal viscometer revealed widely varying electrical conductivities and dielectric permittivities of the mixtures.



Non-ideal viscosity of liquid R32 + Propane (R290) mixtures.

Publications:
Kiselev, S.B., Perkins, R.A., and Huber, M.L. ***"Transport Properties of Refrigerants R32, R125, R134a, and R125 + R32 Mixtures in and Beyond the Critical Region,"*** Int. J. Refrig. 22, 509 (1999).

Laesecke, A., Lüddecke, T.O.D., Hafer, R.F., and Morris. D.J., ***"Viscosity Measurements of Ammonia, R32, and R134a. Vapor Buoyancy and Radial Acceleration in Capillary Viscometers,"*** Int. J. Thermophys. 20, 401 (1999).

19. *Radiation Induced Degradation of PCBs in Various Media*

P. Neta, D.L. Poster (839), and M. Chaychian, J. Silverman and M. Al-Sheikhly (Univ. of Maryland)

Objective: To obtain kinetic and mechanistic information on the radiolytic dechlorination of polychlorinated biphenyls (PCBs) in various media and to develop strategies for detoxification of polluted sites by electron beam irradiation.

Problem: The widespread use of polychlorinated biphenyls in various industrial applications presents a major environmental issue because of the toxicity and long lifetime of these compounds in ambient conditions. The method currently used to destroy most PCB containing materials is incineration. This method, however, suffers from two disadvantages: it incinerates the medium along with the PCB, and it converts some of the PCB into more toxic materials, namely dioxins.

Approach: Radiolytic degradation of PCBs is expected to overcome both of these disadvantages. The PCBs in organic solvents such as transformer oils may be reduced into benign inorganic chloride and practically non-toxic biphenyl, without formation of any dioxins. Such treatment leaves the solvents practically unchanged so that they can be recycled instead of incinerated. This approach may be adapted to removal of PCBs in sediments and soils by combining it with extraction or other treatment methods.

Results and Future Plans: We have examined the radiolytic degradation of several PCBs in water/alcohol mixtures, in micellar aqueous mixtures and in transformer oil. We analyzed the various intermediate and final products, investigated the mechanism of reduction, and determined the rate constants for the relevant reactions by pulse radiolysis. Irradiation of PCBs in oil is not expected to lead to dechlorination by direct reaction of the solvated electrons with the PCB, because the oil contains substantial quantities of other aromatic compounds, which also react with solvated electrons very rapidly. Yet, complete degradation of tetrachlorobiphenyl (PCB 54) in transformer oil was achieved by ionizing radiation (200 kGy for 0.27 mg/g PCB 54). By analyzing samples irradiated with various doses, the gradual degradation of PCB 54 and the successive formation and degradation of trichloro-, dichloro-, and monochlorobiphenyl were demonstrated. The final products were mainly biphenyl and inorganic chloride. The mechanism of reductive degradation in transformer oil was established by pulse radiolysis. Irradiation of the oil leads to production of radical anions from the main aromatic components: biphenyl, fluorene, phenathrene, and pyrene. These radical anions transfer an electron to chlorinated biphenyls relatively rapidly, leading to dechlorination. The rate constants for several such reactions, determined individually in 2-propanol solutions, are found to be in the range of 10^7 to 10^8 L mol^{-1} s^{-1}. Electron transfer between the various polycyclic aromatics is a reversible process for which the equilibrium depends on the reduction potential of each compound. Electron transfer to a chlorinated compound, however, is irreversible because of dechlorination. Thus, the reaction can proceed to complete dechlorination even if the rate constants for dechlorination are relatively low.

Future experiments are aimed at demonstrating complete radiolytic degradation of PCBs in actual oil samples of varying PCB contents and in sediments contaminated with PCBs. Irradiation of solid sediments is expected to have low radiolytic efficiency. Attempts will be made to enhance the efficiency by using aqueous suspensions with various additives and by combining ultrasonic treatment along with the irradiation to enhance the transfer of the PCB into the liquid component, where it will be efficiently dechlorinated.

Publications:
Schmelling, D. C., Poster, D. L., Chaychian, M., Neta, P., Silverman, J., and Al-Sheikhly, M., ***"Degradation of Polychlorinated Biphenyls Induced by Ionizing Radiation in Aqueous Micellar Solutions,"*** Environ. Sci. Technol. 32, 270 (1998).

Chaychian, M., Silverman, J., Al-Sheikhly, M., Poster, D. L., and Neta, P., ***"Ionizing Radiation Induced Degradation of Tetrachlorobiphenyl in Transformer Oil,"*** Environ. Sci. Technol. 33, 2461 (1999).

20. *A Screening Tool for the Environmental Impact of New Fluids*

F. Louis, C. Gonzalez, V. L. Orkin, M. J. Kurylo, and R. E. Huie

Objective: To develop a screening tool, based on quantum mechanics, for the prediction of the environmental impact of new classes of halogenated compounds.

Problem: Experimental studies from this Division have demonstrated that the reactivity of the hydroxyl radical toward halogenated organic compounds is not adequately correlated by simple structure-activity relationships. This was particularly evident when an ether linkage was introduced, where even the order of reactivity could not be predicted. This implied that it would be necessary to measure rate constants for a large number of members of any new class of reactants in order to predict the environmental impact of these possible new solvents, refrigerants, or fire suppressants. It was clear that a new approach was needed.

Approach: The basic idea underlying this project is to establish a level of theory which will predict the reactivity of the hydroxyl radical with a series of simple molecules, at the lowest possible degree of computational difficulty. This level of theory is then applied to more complex molecules and, ultimately, it is applied to the new class of interest. Then it is validated by a limited number of experimental determinations. The reality of the approach is much more complex and multidimensional.

Results and Future Plans: In the initial study, several levels of theory were explored for the reaction of OH with CH_2Br_2. This study included the treatment of tunneling in three different manners. This molecule was chosen both because of the importance of bromine as a fire suppressant and because the relatively large electron system of the molecule makes this reaction a serious test of the various levels of theory. Building on the results of this study, the reactions of OH with the other halogen-substituted methanes, up to bromine, were investigated. From these studies, we chose a level of theory and have been investigating the reactions of OH with several fluoroethanes and the ethers derived from them. These pairs were chosen to represent the extremes of behavior observed experimentally: an increase in reactivity upon addition of the ether linkage; a reduction in reactivity; and a small change in reactivity upon addition of the ether linkage. Theory has been able to reproduce the observed trends, with predictions in absolute reactivity within a factor of three. For bis-(difluoromethyl) ether, we have carried out a more exhaustive theoretical analysis of the reaction surface in order to understand better these reactions in general. At the present, we are further refining this approach, with a particular emphasis on better tunneling corrections and the use of pseudo-potentials. Calculations are being extended up to ethers with several carbons and containing fluorine and one or more bromine atoms. In order to verify these calculations, a sample of 2-bromo-1,1-difluoroethyl methyl ether is being synthesized for us, which we will use for an experimental determination of the rate constant.

Publications:

Orkin, V.L., Villenave, E., Huie, R.E., and Kurylo, M.J., ***"Atmospheric Lifetimes and Global Warming Potentials of Hydrofluoroethers: Reactivity Toward OH, UV Spectra, and IR Absorption Cross Sections,"*** J. Phys. Chem. A (in press).

Louis, F.,Gonzalez, C., Huie, R.E., and Kurylo, M.J., ***"An* Ab Initio *Study of the Reaction of Halomethanes with the Hydroxyl Radical. Part 1: CH_2Br_2,"*** J. Phys Chem. A (in press).

Louis, F., Gonzalez, C., Orkin, V., Huie, R. E., and Kurylo, M. J., ***"An* Ab Initio *Study of the Reaction of Halomethanes with the Hydroxyl Radical. Part 2: CH_2F_2, CF_2FCl, CH_2FBr, CH_2Cl_2, CH_2ClBr, CH_3F, CH_3Cl, and CH_3Br; Reactivities and Infrared Radiative Forcings,"*** J. Phys Chem. A (in press).

21. *Databases for Identification of Chemicals by Gas Chromatography: Natural Gas and Alternative Refrigerant Applications*

T.J. Bruno

Objective: To provide an efficient, fast, and reliable method for identifying a wide variety of chemical compounds in both the laboratory and the field. Initial applications include the heavier components of natural gas (the C_6+ fraction), natural gas treatment materials, and alternative refrigerant fluids.

Problem: The design and operation of many processes involving fluids rely on an accurate chemical analysis of the fluid stream composition. For example, calorific value of natural gas is calculated from a chromatographic analysis of each individual gas stream. Custody transfer of natural gas is therefore based upon a detailed compositional analysis. The most common analysis of natural gas at present considers only the lighter components. This approach introduces significant uncertainty to subsequent calculations based on the gas composition. The inclusion of the heavier fraction into the analysis is a complex problem, because the gas consists of upwards of 400 organic and inorganic constituents. Moreover, the composition varies with season, with source-well long-term history, and with short-term usage and storage history. Thus, a fast, low-cost, and reliable method is required for the efficient commerce and use of this vital natural resource. In the refrigeration industry, the thermal properties of a mixed working fluid is strongly composition dependent. Proper refrigeration machine design and operation therefore depends upon an accurate composition measurement. Not only must the initial filling be at precisely controlled mixture compositions, but also maintenance fillings must be so as well. Because the constituents of mixed working fluids leak at different rates, replenishment maintenance requires an accurate fluid chemical analysis.

Approach: Gas chromatography offers an economical and accurate solution to the problem of natural gas analysis and mixed refrigerant fluid analysis. In addition to being one of the most well understood and economical analytical methods available, it is very amenable to field applications. What is needed is an interactive database that can be incorporated into the control and analysis software of both laboratory and field gas chromatographic instrumentation. We have approached the problem of standardizing and automating these analyses through the measurement of standard chromatographic retention parameters on the most useful stationary phases available, and also on some novel developmental phases. The standard retention parameters that we have measured include net retention volumes, relative retentions, and Kovats retention indices. These parameters are corrected for instrumental variation and are, therefore, reproducible from instrument to instrument. The measurements are performed on a specially modified commercial gas chromatograph that provides highly accurate retention information. The column temperature dependence of each retention parameter is modeled with appropriate equations to allow predictions at all relevant temperatures. These models then form the heart of an interactive database that allows off-line identification of peaks and also the optimization of more complex analyses.

Results and Future Plans: The measurement of all standard retention parameters (at several column temperatures) has been completed for 90 natural gas hydrocarbons, 23 natural gas odorization compounds, and 120 alternative refrigerant fluids on ten useful stationary phases. These stationary phases include the methyl silicones and derivatives, porous polymer and solid adsorbents, and some novel stationary phases that include sol/gel phases and clay phases. Several versions of the databases have been released, and are being used in the gas industry and by regulatory agencies. Next year, we will complete the final versions of the databases, suitable for several computer platforms.

Publications:

Bruno, T.J., Bachmeyer, G.M., and Wertz, K.H., ***"Gas Chromatographic Retention Parameters Database for Refrigerant Composition Management,"*** Int. J. Refrig. 21, 639 (1998).

22. *The NIST Mass Spectral Database: Extending the Evaluation*

S.E. Stein, A. Mikaya, Jane Klassen, Zhu Damo (Guest Researcher), D. Tchekhovskoi (Contractor), C.L. Clifton, and W.G. Mallard

Objective: To provide a fully evaluated mass spectral database with tested and documented search algorithms that will enable the positive identification of unknown organic compounds using gas chromatography/mass spectrometry (GC/MS).

Problem: Modern organic analytical chemistry is critically dependent on instrumental analysis. For qualitative analysis, there is no better tool than the mass spectrometer. Like many other analytical techniques, it is best used with a library of reference spectra. Even with good reference spectra, the data must be processed correctly. Robust algorithms that have been thoroughly tested to eliminate flaws are needed.

Approach: The ongoing work after the release of the NIST mass spectral data base, NIST 98, will add new fully evaluated mass spectra to the database of mass spectra with 129,136 evaluated spectra for 107,886 compounds. New data from laboratory work at NIST, spectra purchased from commercial firms, and contributed spectra are being added. As in prior years, emphasis is being placed on the evaluation of the most important spectra and on improvements of the search software.

Results and Future Plans: The addition of the over 17,000 spectra from the National Institute of Materials and Chemical Research of Japan is an important milestone. These spectra are of the highest quality and are primarily focussed on common compounds. The evaluation has been done using the same techniques established for NIST 98. The data were examined for reasonable neutral losses, for air peaks, for impurities, and for errors in transcription. In every case where a change had to be made, agreement between at least two evaluators was required. The analysis was always conservative; if a given spectrum or spectral feature was not clearly in error, it was not changed. Evaluation is continuing on the spectra obtained from a number of chemical suppliers.

Development of algorithms to aid in the evaluation, as well as to provide users of the NIST software with tools for analyzing spectra from compounds that are not in the database, is ongoing. The software needed for the basic searching has been steadily improved. The new release adds a number of features that make it easier to use the proven NIST algorithms. In addition, the ability to add user-drawn structures and synonyms to the user data has been implemented.

Work is continuing on the addition of retention indices to the database. The retention index is a measure of the time it takes a compound to elute from the gas chromatography column. For many compounds, which might otherwise be confused just using the mass spectrum, the retention index makes it possible for a more definitive positive identification to be made. An example of an important class of compounds for which the retention time data is especially useful is the hydrocarbons, many of which have very similar mass spectra. The first stage of compiling and entering the data has begun. Tools are being developed for both evaluation of the data and for prediction of retention times for compounds for which data are not available.

Publications:

Ausloos, P., Clifton, C., Lias, S.G., Mikaya, A., Sparkman, O.D., Stein, S.E., Tchekhovskoi, D., Zaikin, V., and Zhu, D., ***"The Critical Evaluation of a Comprehensive Mass Spectral Library,"*** J. Amer. Mass. Spect. 10, 287 (1999).

Stein, S.E., Fateev, O.V., Tchekhovskoi, D., Zaikin, V., and Zhu, D, Mikaya, A., Sparkman, O.D. Ausloos, P., Clifton, C., Lias, S.G., Levitsky, A., and Mallard, W.G., ***"NIST/NIH/EPA Mass Spectral Database—NIST 98,"*** Standard Reference Database No. 1, Software Release Version 1.

23. *Automated Gas Chromatography/ Mass Spectral Decomposition and Analysis: Tools for Automating and Improving the Use of GC/MS Instruments*

S.E. Stein, O. Toropov (Contractor), J. Klassen, W.G. Mallard, and J.J. Reed

Objective: To develop and test algorithms for automatically deconvoluting and analyzing GC/MS data files using a target library of compounds.

Problem: The program has been funded by the Defense Threat Reduction Agency (DTRA) to provide a method for analyzing for chemical weapons banned under the Chemical Weapons Convention. The software implementing the algorithms must provide full blinding of the analysis process not to compromise the proprietary data of treaty participants. In general, the manual analysis of GC/MS data files for complex mixtures can be time consuming and error prone. The normal method of doing a background subtraction to extract the single component can be essentially impossible in a complex mixture because there is no background. Even in only moderately complex chromatograms, a manual subtraction can produce seriously erroneous results. In addition, the use of retention information to reduce false positives is far more efficient with computer techniques.

Approach: A detailed noise analysis is performed, followed by a deconvolution of each of the peaks in the total-ion gas chromatogram. The resulting components are then compared to reference spectra using a series of algorithms to emulate the degree of confidence that an analyst would have in the deconvoluted peak. The process of extracting the distinct components out of a complex data file breaks down into four parts: noise perception and evaluation, component perception, signal extraction, and compound identification. The noise perception and evaluation step is central to the analysis because the recognition of the difference between a "real" peak caused by a compound eluting from the column and a "false" peak caused by noise depends upon a knowledge of the nature and size of the noise. Once the noise is understood, the individual components are extracted. The extraction of the signal involves examining the overlap of components and removing mass spectral peaks associated with a different component. Ongoing testing involves a number of laboratories both in the United States and abroad where specific chemical agent samples are examined.

Results and Future Plans: The algorithm was tested extensively by using a target library of chemical weapons agents. Over 40,000 data files were examined to ensure that the algorithm does not produce false positives. At the same time, a number of experiments was performed by other laboratories with low concentrations of chemical agents to demonstrate that the algorithm is sensitive enough to detect true positives at analytically useful concentrations. The results of these tests have shown that the algorithms used in the development of the software are robust and capable of automated and blinded analysis. This year, Version 2 of the software was released. This version includes a number of small changes in the algorithm that resulted from the testing effort as well as the inclusion of the ability to process a number of new instrument file formats.

The use of retention indices is central to the further reduction of false positives. The software developed here has been adapted by the Organization for the Prohibition of Chemical Weapons (OPCW) for use in all inspections involving GC/MS instrumentation. Work to improve the ability to predict retention index data for chemical agents from structural information and from physical property data on analogous compounds is ongoing.

Publications:

S.E. Stein, ***"In Integrated Method for Spectrum Extraction and Compound Identification from Gas Chromatography/Mass Spectrometry Data,"*** J. Amer. Mass. Spect. 10, 770 (1999).

24. *Measurements of Surface Tension of Mixtures*

***C.D. Holcomb**, S.L. Outcalt, and M.O. McLinden*

Objective: To extend significantly the accuracy, the temperature range, and the pressure range of surface tension measurements for mixtures by creating a unique phase equilibrium apparatus with the implementation of a non-visual, non-mechanical method of measuring the surface tension with the simultaneous measurement of the coexisting densities.

Problem: There are three major problems that have prevented the accurate measurement of the surface tension of mixtures over wide ranges of temperature and pressure. The first involves the difficulty of the type of method selected. The more difficult and time consuming the measurement method, the greater are the sources of measurement error. Capillary rise, maximum bubble pressure, Wilhelmy plate, Du Noüy ring, pendant drop, and sessile drop methods all require visual measurements of a height, width, and/or depth to determine accurately the surface tension. These methods require a person or camera and software to make the measurement which can introduce human, optical, or round-off errors. The second problem involves mechanical manipulation of the measurement technique. Capillary rise, Wilhelmy plate, and Du Noüy ring methods all require the measurement device to be submerged in the liquid and then withdrawn. Mechanical manipulations reduce the operating pressure of the system and add experimental complexity to the measurement of the surface tension. Finally, all methods of measuring the surface tension require knowledge of the densities of the coexisting liquid and vapor phases. Currently, the densities are either estimated or calculated from equations of state. By not measuring the densities directly, especially for more complex mixtures, uncertainties in the density prediction increase the uncertainty in the surface tension.

Approach: All three of these major problems have been eliminated in our approach which uses the differential-bubble pressure method of measuring surface tension and two vibrating tube densimeters for measuring the coexisting densities. These are incorporated into a dual-recirculation-loop high-pressure phase equilibrium apparatus that operates between 223 K and 423 K. The differential bubble pressure method is a variation of the maximum bubble pressure method that eliminates the need for a visual measurement of the depth of submersion of the dip tube. Two dip tubes of different radii are submerged in the liquid to the same depth. The difference in the maximum bubble pressures of the two dip tubes is related to the surface tension, but the pressure effect determined from the depth of submersion of the tubes is canceled. Second, the method does not require any mechanical manipulations. The tubes are mounted in a fixed position and only require that the liquid level is high enough to cover the ends of the tubes. Finally, vibrating tube densimeters are mounted in the two recirculation loops of the phase equilibrium apparatus and are used to measure the densities of the coexisting phases. This eliminates the need for predictions or an equation of state to estimate the densities. The temperature, pressure, and compositions of the phases are recorded as part of the basic phase equilibrium measurement.

Results and Future Plans: The surface tensions and densities of pure isopentane, n-hexane, three mixtures of R32/125, three mixtures of R143a/125, two mixtures of R245fa + isopentane, two mixtures of R123 + isopentane, and two mixtures of R123 + n-hexane have been measured in this apparatus from 280 K to 340 K at pressures to 3.2 MPa. These measurements were used to develop a Moldover-Rainwater model for the surface tension of mixtures. Preliminary evaluation of the modified prediction method show better agreement with the experimental data for a wider range of fluids than for the original model. Another advantage of the modified prediction method is that it does not depend on an equation of state to calculate the fugacity fraction. A final advantage of the modified prediction method is that a single interaction parameter can be added to the model to increase the accuracy of the prediction and allow for more accurate predictions for a wider range of systems. In the future we will perform measurements on natural gas mixtures, aqueous/solvent mixtures, and lubricants.

25. *Modeling of Molecular Systems: Thermodynamics, Void Volumes, and Solid-Liquid Equilibrium*

*J.C. **Rainwater**, P.D. Beale (Univ. of Colorado), and S.G. Gay (Univ. of Colorado)*

Objective: To develop a molecular theory of solid-liquid equilibrium (SLE) for pure molecular fluids and mixtures, including hydrocarbons, refrigerants, and polar fluids, and to develop techniques for modeling molecular systems in supercooled liquid, glassy, and amorphous states.

Problem: The complete description of a pure fluid or mixture requires knowledge of the fluid-solid boundary. Our SLE research to date indicates that molecular shape is important and that theories restricted to spherical molecules need to be generalized to nonspherical molecules. There exist methods to determine the thermodynamic properties of hard-sphere systems in terms of average void volumes and surface areas, which we are extending to elongated molecules.

Approach: For SLE, we have followed the approach of P.A. Monson of the University of Massachusetts. The molecule is modeled as a fused hard sphere assembly, for example, a homonuclear hard dumbbell for nitrogen or a heteronuclear dumbbell for methyl chloride. For the hard-body system, the solid free energy is determined in a computationally intensive manner by the method of Frenkel and Ladd, in which at least ten simulations must be performed. More theoretically, the free energy is calculated by the Lennard Jones-Devonshire cell model, from the free volume of a test molecule in a cage of fixed neighboring molecules on a lattice.

The liquid free energy is obtained by simulation, and the phase boundary is determined by the double-tangent construction. At the end, mean-field attractive forces and dipole and quadrupole moments are added as perturbations. Free volumes and surface areas are monitored, and expressions are derived for the system pressure in terms of average free volumes and surface areas.

Results and Future Plans: After completion of our study of methyl chloride, we turned our attention to a fluctuating cell theory and the relationships between pressure and void properties for two-dimensional hard dumbbells. The fluctuating cell theory differs from the simple cell theory in that the positions of the cage molecules are allowed to fluctuate, and an average free volume is calculated. The fluctuating cell theory was expected to give better agreement with the results of the full Frenkel-Ladd calculation with multiple simulations than does the simple cell theory. We found this to be true for all hard dumbbells except when the bond length is very small, in the limit of a hard disk. In that limit, we recovered the earlier, counterintuitive discovery of Hoover *et al.* that the fluctuating cell theory gives poorer agreement than the simple cell theory. We have compared our earlier exact solution for void volumes in three dimensions of a hard-sphere system with a newly published, independent solution of the same problem by a group from Princeton and Bell Labs. Numerical results from the two solutions have been shown to be identical. An important remaining goal is to calculate free volumes of three-dimensional hard dumbbells semi-analytically, where the spatial dimensions are integrated analytically and the two angular variables are integrated by polynomial quadruture. At present we have a robust algorithm for the semianalytical method, but it is slower than Monte Carlo. However, there are a number of possible ways of making the semianalytic method substantially faster, and these will be pursued. If successful, the method will allow for a fluctuating cell model in three dimensions with applications to plastic crystals and more complex molecules. Our first planned extensions are to triatomics with dipole moments, such as sulfur dioxide, and molecules that can be approximately modeled as three fused spheres, such as dimethyl ether and propane.

26. *Thermophysical Properties of Partially Characterized Systems*

M.L. Huber, D.G. Friend, C.D. Holcomb, S.L. Outcalt, and J.R. Elliott (Univ. of Akron)

Objective: To provide industry with models for the thermophysical properties of partially characterized systems such as petroleum fractions and lubricants.

Problem: One may encounter fluid systems that cannot be easily characterized in terms of known compositions of pure fluids. An example is a petroleum fraction, where often the fraction is characterized by an average boiling point and a density or specific gravity, and the exact composition of the fluid is not known. Another example of a partially characterized system is a lubricant. These systems are typically proprietary mixtures generally characterized by their viscosity and density. Currently there is a lack of models for the thermophysical properties of these types of systems. In the refrigeration industry, the lack of adequate models for mixtures of alternative refrigerants and lubricants prevents optimal equipment design.

Approach: We are pursuing two different approaches that build upon existing work performed at NIST. For petroleum fractions, we are developing models based on the theory of extended corresponding states that has been shown to be reliable for nonpolar hydrocarbons of low-to-moderate molar masses. For synthetic lubricant systems, we are investigating the use of various equations of state such as statistical associating fluid theory (SAFT) and Elliott, Suresh and Donahue (ESD). In addition to these models, we plan to model the mixture with a Helmholtz-energy-based mixture model that has been very successful in modeling the thermophysical properties of refrigerants and refrigerant mixtures. This allows us to build upon and enhance existing NIST databases such as NIST4 (Thermophysical Properties of Hydrocarbon Mixtures) and NIST23 (REFPROP: Thermodynamic Properties of Refrigerants and Refrigerant Mixtures).

Results and Future Plans: We have developed an upgrade to the NIST4 (SUPERTRAPP) database that allows computation of the thermophysical properties of undefined petroleum fractions (only API gravity and an average boiling point are required). The new version is currently undergoing review by Standard Reference Data, and we anticipate the public release of this new version in the very near future.

We began our refrigerant/lubricant work with some preliminary bubble-point measurements on the R134a/lubricant system. We applied the ESD equation of state and made comparisons with our own data and with literature data. The results of the work so far are promising, but it is only a first step in the analysis of refrigerant/lubricant systems. As more data become available for the lubricant, we will further develop our models for the lubricant. Future work on the mixture model will focus on developing mixing and combining rules. The eventual goal is to incorporate lubricants into the REFPROP database.

Publications:

Huber, M.L., Holcomb, C.D., Outcalt, S.L., and Elliot, J.R., ***"Vapor-Liquid Equilibria for a R134a/Lubricant Mixture: Measurements and Equation-of-State Modeling,"*** ASHRAE Trans. (in press).

Huber, M.L., ***"NIST Standard Reference Database 4: NIST Thermophysical Properties of Hydrocarbon Mixtures, Version 3.0,"*** National Institute of Standards and Technology, Standard Reference Data Program, Gaithersburg, MD (1999).

27. *Behavior of Fluid Systems Under Shear: Characterization and Metrology*

C. D. Muzny and H.J.M. Hanley

Objective: To understand the relationship between fluid properties and shear and subsequently to control nanoscale structural properties through the application of shear.

Problem: The flow properties of a complex system can be predicted or controlled if the relationship between its structure and rheological characteristics is understood. We argue that the structure can be determined by radiation scattering, but that such data must be correlated with rheological data obtained simultaneously from a rheometer or viscometer.

Approach: We recently modified a constant stress rheometer adapted so that a Couette cell can be placed in the neutron beam. The apparatus is capable of high accuracy measurements with viscosities that can range over about ten orders of magnitude. This apparatus is a powerful, generic contribution to the metrology required to investigate systems out of equilibrium. We have also adapted our light scattering equipment to extract dynamic information from a particular shear-modified gelling sample. The results from all of these experiments are coordinated with computer simulation data of model systems under shear.

Results and Future Plans: Studies with our neutron scattering adapted rheometer include correlating the alignment of macromolecules with their viscosity in solution and extensive investigations relating structure changes with viscometry of gelling colloidal silica. Our light scattering system has recently be used in studies of gelling systems under oscillatory shear, the results of which can lead to a better understanding of the chemistry of the process. Computer simulation results on the morphology and structure factor of a two-dimensional system of particles interacting through a Lennard-Jones potential, modified to include a long-range repulsive component, have also been reported. It was shown that gel formation can be regarded as the competition between the short-range attractive forces (cause aggregation) and the long-range repulsion forces (keep particles separate) that encourage the formation of space-filling networks.

Future plans include extending our investigations to x-ray scattering, and to extend the current small angle neutron and light facilities. If successful, we will then have the potential not only to investigate materials over the wide range of length scales, which is our objective, but also will have the flexibility to investigate systems with the most appropriate scattering tool.

Publications:

Straty, G.C., Muzny, C.D., Butler, B.D., Lin, M.Y., Slawecki, T.M., Glinka, C.J., and Hanley, H.J.M., ***"An in situ Rheometric Shearing Apparatus for SANS,"*** Physica B: Condens. Matter 241-243, 74 (1998).

Butler, B.D., Muzny, C.D., and Hanley, H.J.M., ***"Scaling of Small-Angle Neutron Scattering Intensities from Gelling Colloidal Silica,"*** Int. J. Thermophys. 20, 35 (1999).

Hanley, H.J.M., Muzny, C.D., Butler, B.D., Straty, G.C., Bartlett, J., and Drabarek, E., ***"Shear-Induced***

***Restructuring of Concentrated Colloidal Silica Gels*,**" J. Phys: Condens. Matter 11, 1369 (1999).

Butler, B.D. and Hanley, H.J.M., ***"Aggregation in Quenched Systems Interacting via a Short-Range Attractive, Long-Range Repulsive Potential,"*** J. Sol. Gel Sci. and Tech. 15, 161 (1999).

Muzny, C.D., Butler, B.D., Hanley, H.J.M., and Agamalian, M., ***"An Ultra-Small-Angle Neutron Scattering Study of the Restructuring of Sheared Silica Gels,"*** J. Phys: Condens. Matter 11, L295 (1999).

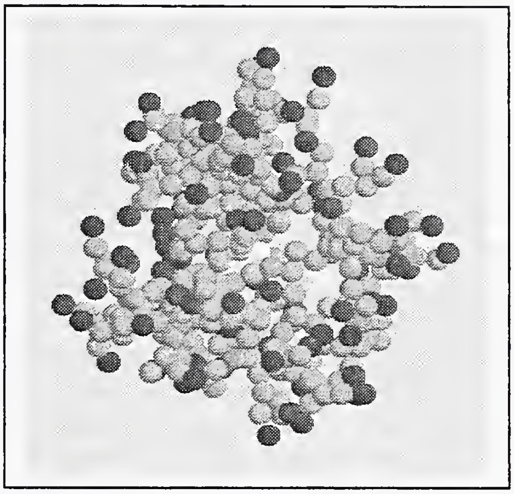
28. *Theory and Simulation of Complex Fluids*

L. Lue, B.D. Butler, D.J. Evans (Australian Natl. Univ.), L.V. Woodcock (Univ. of Bradford), and S. Gay (Univ. of Colorado)

Objective: To develop new, more efficient computer simulation methods for complex fluid and solid systems; to improve our understanding of single component and multi-component atomic and molecular models, both in and out of equilibrium; and to improve current predictive models of fluid properties through a better understanding of model systems.

Problem: In general, computer simulation is not effective at predicting, from first principles, the behavior of real fluids with the accuracy required by chemical engineers. This is not only because of current limits on computational resources; the available simulation methods and algorithms also need to be improved. However, by providing an "ideal" laboratory in which to study the behavior of systems containing a large number of interacting particles, computer simulation provides an important tool in the study of the thermophysical properties of complex fluid and solid systems. This "ideal" laboratory allows precise testing of theories for real systems. In addition, computer simulation yields insights into the fundamental nature of the structure and dynamics of complex systems. These insights, for example, have been incorporated into semi-empirical equations that are used for the prediction of fluid properties in technologically important systems. The improvement and application of computer modeling algorithms are thus essential for progress in the development of prediction tools required by industry.

Approach: Our simulation and modeling activities concentrate on areas of current interest in the Division. Some examples include the process of aggregation in quenched systems as models of gelation phenomena, the effects of shear on the thermodynamic states of fluids, steric effects in binary systems and their implications on solid-fluid equilibrium, and the behavior of macromolecules. By identifying and isolating weaknesses in current methods and theories, alternatives are developed, tested, and improved.



Results and Future Plans: Aggregation phenomena have been investigated in systems with potential functions that contain a short-range attractive and long-range repulsive component. These systems reproduce many interesting effects observed in real aggregating systems, such as network formation. A crossover theory for the structure and thermodynamics of linear and star polymers in good solvents has been developed and tested using Monte Carlo methods. This theory is able to describe the scaling behavior of dilute to semidilute polymer solutions, as well as the properties of concentrated polymer systems. Monte Carlo studies have also been performed for dendritic polymer solutions (cf. figure). Methods to study depletion forces that arise from entropic considerations in binary hard-sphere systems have also been developed. The concept of "configurational temperature," which was previously developed and tested for atomic systems, has been extended to molecular systems. A generalization of the Poisson-Boltzmann equation that accounts for nonelectrostatic interactions has been developed. Future plans include the incorporation of the concept of configurational temperature to the thermostatting of nonequilibrium molecular dynamics models, the study of shear on aggregation processes, the simulation and modeling of glassy systems, and the testing of theoretical predictions of phase equilibria in binary mixtures.

Publication:
Lue, L. and Kiselev, S.B., ***"Crossover Approach to Scaling Behavior in Dilute Polymer Solutions: Theory and Simulation,"*** J. Chem. Phys. 110, 2684 (1999).

29. *Molecular Dynamics Examination of Microheterogeneity in Liquids*

R. D. Mountain

Objective: To develop models that describe the size of microheterogeneous regions (on molecular length scales) in supercritical fluids and in aqueous mixtures.

Problem: Reactions involving solutes can be significantly influenced by the presence of void regions (supercritical fluids) or by compositional heterogeneities (aqueous mixtures) if the solutes are nonuniformly distributed in the fluid. Knowledge of when and where such nonuniformities occur and how different solutes are partitioned by such environments is lacking and limits our ability to predict and optimize reactions.

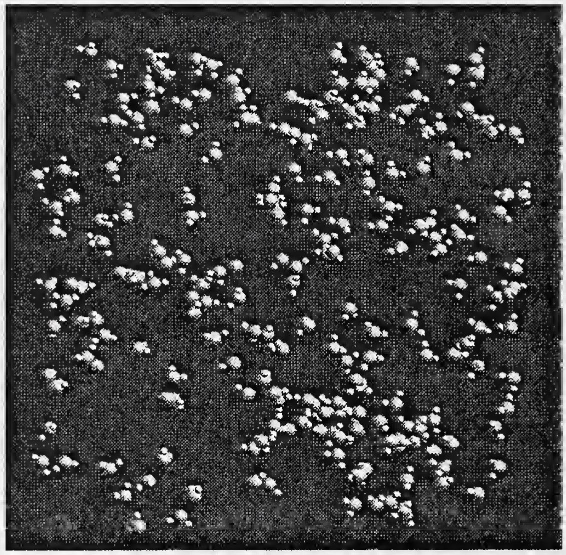
Approach: Molecular dynamics simulations of water, carbon dioxide, acetonitrile, and water-acetonitrile mixtures have demonstrated that existing model potentials for these fluids provide good descriptions of thermal properties. Simulations are being used to determine the size of clusters and voids in supercritical water and in supercritical carbon dioxide. Simulations are also being used to determine the structure of compositional heterogeneities in water-acetonitrile liquid mixtures over a wide range of compostions and temperatures.

Results and Future Plans: Now that the conditions where microheterogeneity occurs for these fluids are known, it is a straightforward task to introduce various solutes into the system.

The simulations are being used to examine the solvation and degree of association of ions in water for ambient and supercritical conditions. Related studies of ions in water-acetonitrile mixtures will be made. Also, the solvation of polar and nonpolar solutes will be examined for the mixtures and for supercritical fluids that relate to experiments underway in the Division.

Publications:
Mountain, R. D., ***"Voids and clusters in expanded water,"*** J. Chem. Phys. 110, 2109 (1999).

Mountain, R. D., ***"Molecular dynamics study of water acetonitrile mixtures,"*** J. Phys. Chem. B (in press)



A snapshot of supercritical water showing the clustering that dominates the structure of the fluid.

30. *Primary Acoustic Thermometry*

D. Ripple (836), M.R. Moldover, and K.A. Gillis

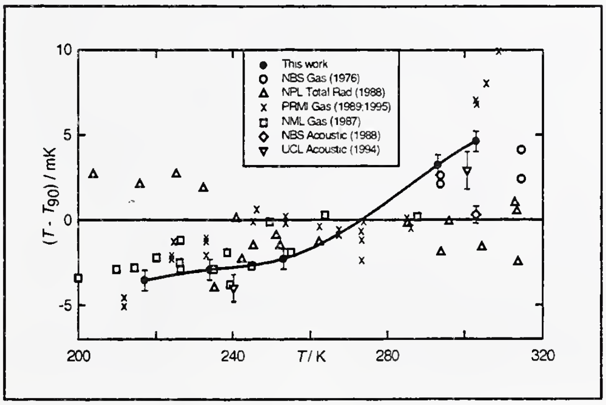
Objectives: (1) To reduce the uncertainty in the determination of the thermodynamic temperature by a factor of 3 to 8 in the range from 500 K to 900 K using speed-of-sound measurements in low density argon as a primary standard and (2) to improve the accuracy of the high-temperature fixed points (*e.g.* tin point, zinc point) and radiometry tied to these fixed points.

Problem: The most accurate determinations of thermodynamic temperature above 700 K use relative radiance measurements referenced to a black body near 700 K. The thermodynamic temperature of the black body is known from NIST constant volume gas thermometry (CVGT) experiments. Unfortunately, two NIST CVGT experiments differ from each other for reasons that are not well understood. The difference leads to an estimated uncertainty of 13 mK in temperatures near 700 K and 50 mK in temperatures near the gold point (1337.33 K).

Approach: We measure the frequencies of both acoustic and microwave resonances in a spherical, argon-filled cavity bounded by a thick, metal shell, enclosed by a high-performance thermostat. The data determine the speed of sound in the argon from which the thermodynamic temperature is deduced. The temperature is transferred to platinum resistance thermometers and then to fixed-point devices.

Results and Future Plans: Microwave and acoustic data in the temperature interval 217 K to 303 K were acquired with a prototype resonator. These data determined ($T - T_{90}$), the difference between the Kelvin thermodynamic temperature T and the International Temperature Scale of 1990 (ITS-90) with a standard uncertainty of 0.6 mK, depending mostly upon the model fitted to the acoustic data. The graph compares these data with results from other laboratories.

These results were recognized with the "Best Oral Presentation" Award at the 7th International Symposium on Temperature and Thermal Measurements in Science and Industry. The work with the prototype resonator led to many improvements in the high-temperature apparatus.



During the past year, acoustic resonances were successfully measured at 250 °C, in static argon and also in flowing argon. The latter indicates that the purity of the argon can be maintained at high temperatures. It appears that the performance targets for this apparatus will be met, if not exceeded.

Publication:
Moldover, M.R., Boyes, S.J., Meyer, C.W., and Goodwin, A.R.H., ***"Thermodynamic Temperatures of the Triple Points of Mercury and Gallium and in the Interval 217 K to 303 K,"*** J. Res. Natl. Inst. Stand. Technol. 104, 11(1999).

31. *Advanced Refrigeration Systems for Cryogenic Applications*

R. Radebaugh*, P.E. Bradley, E.D. Marquardt, M.A. Lewis, and J.D. Siegwarth; I. Ruelich and H. Quack (Technical Univ. of Dresden); M. Hill (Univ. of Colorado); and J. Gary and A. O'Gallagher (891)*

Objective: To use measurement and modeling techniques for evaluating and improving performance of cryocooler components, such as heat exchangers and pulse tubes and to develop new and improved refrigeration and heat transfer processes for the temperature range below about 230 K.

Problem: Cryocoolers are required for many technology areas, including the cooling of infrared sensors for surveillance and atmospheric studies, the cooling of superconducting electronics and magnets, the cooling of cryopumps for clean vacuums in semiconductor fabrication processes, and the liquefaction of natural gas. The use of these technologies has been hampered because of problems with existing cryocoolers. These problems include short lifetimes, inefficiency, high cost, and excessive vibration. Improved cryocoolers would stimulate the growth of all these technology areas. Proper measurements need to be identified that will characterize losses within these cryocoolers, and models need to be developed to optimize the design of such systems.

Approach: Precision moving parts in existing cryocoolers are a source of wear, vibration, and high cost. Our approach in the development of improved refrigeration processes has been to focus our measurements and modeling on processes that eliminate most, or all, moving parts while still maintaining high efficiency. Much of our research has been on pulse tube refrigerators, which have no cold moving parts. Our studies encompass measurements and modeling of losses to further improve efficiencies of these cryocoolers while increasing their lifetimes. NIST research in this area has much industry and other government agency support to aid in the transfer of this technology to industry.

Results and Future Plans: During FY99, measurements were made on the thermal conductance of packed stainless steel spheres. The thermal conductance degradation factor was found to be 0.11, in good agreement with the value of 0.10 for stainless steel screen. From measurements at various helium filling pressures we have determined that most of the heat is transported by the helium gas a distance of about 4 μm across the boundary rather than by the direct metallic contact. Almost no prior data existed for the thermal conductances of these packed materials, but they are needed for the optimum design of regenerators in many types of cryocoolers. A new *in situ* measurement technique was developed this past year that gave nearly the same result for a regenerator with packed stainless steel screen.

A pulse tube liquefier prototype was completed for NASA/Johnson as part of a program for developing the technology of liquefying oxygen on Mars. NASA expects to have a satellite sent to Mars in the year 2007 that would convert the carbon dioxide atmosphere of Mars into oxygen and then be liquefied using our pulse tube liquefier technology. After two years, a sufficient quantity of liquid oxygen would be collected to fire rockets for lifting off of Mars and returning to Earth with Mars rock samples. Extensive measurements with this liquefier prototype were completed and the results were used to update our models of pulse tube refrigerators. Several types of losses were identified and measured to explain the very high efficiency. The Carnot efficiency of 20 % with respect to input PV power is among the best ever achieved in a cryocooler of this size. This excellent performance indicates the power of the NIST modeling and optimization tools to advance cryocooler technology.

Under sponsorship from the Air Force and the National Radio Astronomy Observatory (NRAO), a program was begun to understand better the thermodynamic and heat transfer processes within regenerators and pulse tubes when operated at high frequencies (< 25 Hz) and temperatures as low as 10 K. Typically, regenerators have had difficulty operating under these conditions and, as a result, have hampered the development of high efficiency and high reliability cryocoolers for temperatures below about 30 K. NRAO and the Air Force have need of such cryocoolers.

32. *Standards for Cryogenic Flow*

J.L. Scott, M.A. Lewis, and J.D. Siegwarth

Objective: To maintain the national standard for cryogenic flow measurement and to advance our services by improving data acquisition, piping configuration, process control, and by reducing measurement uncertainty.

Problem: The cryogenic flow calibration facility is the only independent facility of its kind in the world. It provides the measurement standard for liquefied air gases, and it can be used to evaluate metering methods for liquefied natural gas (LNG), as it becomes a viable alternative fuel. A dynamic weighing system is used to measure the total mass and, with the use of thermophysical property data for density, the volume. Calibrations are typically performed using liquid nitrogen over a flow range of 0.95 kg/s to 9.5 kg/s, a pressure range of 0.4 MPa to 0.76 MPa, and a temperature range of 80 K to 90 K. The precise measurement of any fluid is difficult due to the variability in numerous process conditions; with roughly 570 L of liquid nitrogen at 80 K, the complexity of the measurement is substantially increased. The flow measurement uncertainty is a combination of uncertainties in mass, time, temperature, pressure, and, if volume flow is required, an equation of state for density calculations. While the uncertainty of mass flow measurement is 0.17 %, our uncertainty in volume flow measurement has been three times larger. The new equation of state for nitrogen will make the volume flow measurement uncertainty virtually the same as that of mass flow. Our attention is also focused on mechanical aspects; we must anticipate and prevent component failures due to age and temperature extremes. We want the flow entering the meter under test to be as reproducible as possible, and we must keep the operation and control software on a current platform and in a form that is adaptable to future system operations.

Approach: Improvements are made on a continuing basis to the operation and reliability of the cryogenic flow facility. All components of the weighing system must be in thermal equilibrium with the liquid nitrogen, and, for that reason, measurements are made dynamically. We have upgraded our load cell, the primary measurement instrument in the process, and acquired a high-speed, high-accuracy digital voltmeter to read it. The result is improved reproducibility. The most difficult process control is temperature control. Almost the entire apparatus is vacuum-jacketed with soldered copper piping. As age and thermal cycling compromise these lines, they are replaced by standard stainless steel vacuum piping with o-ring joints. This makes access and modifications simpler. To minimize the effect of flow disturbances upstream of the test section, we have positioned as much straight pipe upstream of the meter installation, as our laboratory space will allow; however, this enhances the problems of pipe contraction at cryogenic temperatures. The inside lines contract, but the vacuum jacket does not. A robust bracing system and a combination of bellows were designed to compensate for these contractions. In the 1980's a minicomputer was installed for data acquisition of the many sensors throughout the system. Though the best choice at the time, operation was cumbersome and difficult. A PC with a graphical programming language now performs the data acquisition. This not only enhances programming flexibility, but it makes it easier to train additional facility operators. This software incorporates a new equation of state for nitrogen as well as the equation that is currently in NIST 12. The new equation will become the NIST standard when the next version of NIST 12 is released (FY00).

Results and Future Plans: We calibrate and/or test various types of flowmeters (turbine, Coriolis, positive displacement) for customers that include meter manufacturers, state regulatory agencies, and aerospace companies. These meters may be used for transfer standards or in test stands. Our independent cryogenic flow calibration facility can help meter manufacturers compete in the international marketplace by providing interlaboratory comparisons with privately held foreign facilities. Continuing improvements to the facility include reducing heat leaks, expanding the flow range of the facility, enhancing automation, and evaluating our processes to ensure that the national cryogenic flow standard is state of the art.

VI. Analytical Chemistry Division (839)

Willie E. May, Chief

A. Division Overview

Analytical Chemistry

W. May, Chief

The Analytical Chemistry Division serves as the Nation's reference laboratory for chemical measurements and standards to enhance U.S. industry's productivity and competitiveness, assure equity in trade, and provide quality assurance for chemical measurements used for assessing and improving public health, safety, and the environment. The Division's activities primarily contribute to the accomplishment of CSTL's measurement science and measurement standards goals through the efforts of its five Groups:

- Spectrochemical Methods,
- Organic Analytical Methods,
- Gas Metrology and Classical Methods,
- Molecular Spectrometry and Microfluidic Methods, and
- Nuclear Analytical Methods.

The skills and knowledge derived from laboratory-based research concerning the phenomena that underpin the measurement of chemical species in a broad spectrum of matrices are applied to the development and critical evaluation of measurement methods of known accuracy, sensitivity, and uncertainty.

Programs

This expertise in chemical measurement science is deployed in a highly leveraged manner and serves as the foundation for cross-cutting chemical measurement and standards programs in:

- Advanced Materials Characterization
- Analytical Instrument Performance and Calibration
- Environmental Monitoring and Technology
- Forensics
- Healthcare
- Food

Our vision is to maximize this leverage by establishing and maintaining the chemical measurement infrastructure that provides national traceability and is the basis for assessing and improving international comparability, for chemical measurements in these and future high priority program areas. To realize this vision we use and develop infrastructural tools such as:

- Standard Reference Materials
- NIST Traceable Reference Materials (NTRMs)
- Measurement quality assurance programs in critical national areas
- Comparisons of NIST chemical measurement capabilities and standards with other National Metrology Institutes.

Increased requirements for quality systems documentation for trade and effective decision-making regarding the health and safety of the U.S. population have increased the need for demonstrating "traceability-to-NIST" and establishing a more formal means for documenting measurement comparability with standards laboratories of other nations and/or regions. Standard Reference Materials (SRMs) are certified reference materials (CRMs) issued under the National Institute of Standards and Technology trademark. These are well-characterized using state-of-the-art measurement methods and/or technologies for chemical composition and/or physical properties. Traditionally, SRMs have been the primary tools that NIST provides to the user community for achieving chemical measurement quality assurance and traceability to national standards.

Currently, NIST catalogs nearly 1300 different types of SRMs; in 1999 NIST sold over 33,000 SRM units to approximately 6,550 unique customers. Approximately 21,000 of the units sold were from the 850 different types of materials that are certified for chemical composition. Since it has the world's leading, most mature, and most comprehensive reference materials program, most of the world looks to NIST as the *de facto* source for high quality CRMs to support chemical measurements. NIST has met the reference materials needs of U.S. industry and commerce for nearly 100 years. While our reference materials program has focused primarily on U.S. requirements, it is clear that these materials address international measurement needs as well. As demonstration of quality and "traceability" for chemical measurements have become increasingly global issues, the need for internationally recognized and accepted CRMs has increased correspondingly. Their use is now often mandated in measurement/quality protocols for analytical testing laboratories. Coupled with the fast pace of technological change and greater measurement needs, the demand for additional quantities and additional specific varieties of reference materials has mushroomed. NIST, by itself, does not have the resources to provide SRMs (exact sample types, unique compound combinations, concentrations, etc.) to meet all these needs. Without a significant shift in paradigm, CSTL will not to be able to address future needs for reference materials; neither nationally nor internationally.

The NIST Traceable Reference Materials (NTRM) program was created to partially address this problem of increasing needs for reference materials with a well-defined linkage to national standards. An NTRM is a commercially produced reference material with a well-defined traceability linkage to existing NIST standards for chemical measurements. This traceability linkage is established via criteria and protocols defined by NIST and tailored to meet the needs of the metrological community to be served. The NTRM concept was implemented initially in the gas standards area to allow NIST to respond to increasing demands for high quality reference materials needed to implement the "Emissions Trading" provisions of the Clean Air Act of 1990 (while facing the reality of constant human and financial resources at NIST). The program has been highly successful. Since its inception, 11 specialty gas companies have worked with NIST to certify over 7000 NTRM cylinders of gas mixtures that have been used to produce more than 475,000 NIST-traceable gas standards for end-users. According to Stephen Miller, Technical Director, Scott Specialty Gases, "the NTRM program has served as an excellent vehicle for production of the high quality standards - of known pedigree - required by both industry and the regulatory community in the implementation of Title IV [SO_2 emissions trading] of the 1990 Clean Air Act." The NTRM model for the commercial production of reference materials is being extended to other mature and high volume areas to both more effectively deliver the increasing number of NIST-traceable standards to end users and allow more of our internal resources to be diverted to address new and/or more difficult measurement problems. Immediate plans are to provide NTRMs for optical filter standards, (Spring 2000) elemental solution standards (Summer 2001), and metal alloy standards (Fall 2001). The term NTRM has been trademarked and we are in the process of obtaining a service mark to facilitate its appropriate use by commercial standards producers.

We recognize that the NTRM model is only applicable in selected areas. In the remaining areas, such as complex matrix standards, we have begun leveraging our resources through increased strategic collaborations with other National Metrology Institutes and selected U.S. laboratories. Additional details concerning our collaborative activities with both private sector U.S. laboratories and commercial reference material and proficiency testing service providers as well as other National Metrology Institutes worldwide are provided in Technical Activity Reports 12 – 18 .

International agreements and decisions concerning trade and our social well-being are increasingly calling upon mutual recognition of measurements and tests between nations. The absence of such mutual recognition is considered to be a technical barrier to trade, environmental and health-related decision making. In recent years, mutual recognition agreements have been established related to testing and calibration services and in respect of the bodies accrediting such activities. All of these rest upon the assumption of equivalence of national measurement standards and reliability of the link between national measurement standards and the relevant testing services in each country. In October, 1999, the Directors of National Metrology Institutes for the thirty-eight member states of the Meter Convention signed "the mutual recognition arrangement on national measurement standards and calibration and measurement certificates issued by national metrology institutes (MRA). This MRA had been in the works for sometimes and with this knowledge, we began taking a leading role in the activities of the International Committee of Weights and Measures-Consultative Committee on the Quantity of Material (CCQM) and Interamerican System for Metrology (SIM) – Chemical Metrology

Working Group. The CCQM activities are focussed on assessing, improving and documenting the equivalence of the chemical measurement capabilities among National Metrology Institutes worldwide. Our activities within SIM are focussed primarily on assessing chemical measurement needs and capabilities among the 34 member economies. We are working with CITAC (Cooperation in International Traceability in Analytical Chemistry) to harmonize the vertical traceability links in the various countries and regions around the world. Additional details concerning these activities can be found in Technical Activity report 18.

In addition to these global and regional activities we are establishing a limited number of strategic bilateral programs. For example, our collaboration with the Netherlands Measurement Institute (NMi) for determining the equivalence of primary gas standards has resulted in a formal "Declaration of Equivalence" that is recognized by the U.S. EPA and European environmental regulatory bodies as documenting the equivalence between seven NIST and NMi primary gas mixtures suites. Details concerning this activity can be found later in the Gas Metrology and Classical Methods section of this overview and in Technical Activity Report 18. We have a formal agreement with NRC-Canada (via the NAFTA Treaty) for cooperation in the development of marine environmental standards and have recently signed a Cooperative Arrangement with NIMC (Japan) for collaborative efforts in the area of pure volatile organic compound standards. Several additional strategic bilateral arrangements with other National Metrology Institutes /Standards laboratories are being discussed.

In addition to our standards activities (SRM, NTRM and strategic international comparisons), providing chemical measurement quality assurance services in support of other Federal and State government agency programs continues to be an important part of our measurement service delivery portfolio. During the past year, we were involved in 13 projects with 6 federal and state government agencies primarily providing them with measurement quality assurance services for environmental and health-related activities. Details concerning many of these have been provided in past Division overviews. Additional information concerning a relatively new activity--QA Support for Private Sector Provision of Proficiency Testing for EPA/States Drinking and Waste Water programs is mentioned later in two of the Group Sections and Technical Activity Report 17.

Brief overviews of each of the five Technical Groups within the Division follow along with 18 selected Technical Activity Reports. These overviews and reports demonstrate the diversity and breadth of our research program and the services that we provide. They also illustrate the critical synergistic relationships that exist among our research programs and activities in chemical measurement science and the standards and quality assurance services that we provide.

Spectrochemical Methods Group

Research activities in spectrochemical methods are directed toward the development, critical evaluation, and application of techniques for the identification and measurement of inorganic species using x-ray, optical, and mass spectrometries. The focus of this research is measurement accuracy and precision, benchmarking industry's needs for traceability, advanced materials characterization, and commodity value assignment and specification.

The major event of the year was the move to the Advanced Chemical Sciences Laboratory. The move of the Group to the 3rd floor of the ACSL results in a consolidation of offices and laboratories from the 1st, 2nd, and 3rd floors of the old Chemistry Building, and the basement of the Physics Building. This consolidation should allow more cohesive and coordinated work by the group, in addition to providing better functionality—sample handling laboratories and instrument laboratories are now contiguous. The move also forced an assessment of the future directions for the Group and a consolidation of instrumentation. Thermal ionization mass spectrometers (TIMS) were loaned to the Physics Laboratory, several university laboratories, DoE, and the Institute for Reference Materials and Measurement (IRMM) in Belgium. The move punctuated the evolution away from solid source mass spectrometry to inductively coupled plasma mass spectrometry (ICP-MS) for the Group. We now have five ICP-MS; and, only two of the NBS-style TIMS instruments remain.

The X-Ray Fluorescence (XRF) team had a number of accomplishments to its credit this year, in both the measurement and research areas. A collaborative effort with a guest researcher from the Naval Research Laboratory to study and compile XRF fundamental parameters is reported as a technical highlight elsewhere in this volume. Progress continued on a collaborative project with a second guest researcher from Poland studying microbeam XRF. A significant amount of SRM work was also completed. Homogeneity testing was done for about a dozen materials in preparation. The Pb paint films of SRMs 1570 –1576 were re-certified with XRF played a major role in this certification effort. Three cement SRMs were certified—starting a major re-certification program for these important and popular SRM materials. The cement SRM category was the only SRM category that showed increased sales for this year. SRM 1848, Lubricant Additive Package, is a new SRM under development. This SRM is top priority of the lubricants industry and is needed to satisfy their requirements for linking measurements made on finished products to recognized national or international standards. Activities leading to the development of SRM 1848 are described in more detail in Technical Activity Report 11.

The high performance ICP-OES research and measurement effort continues to impact a wide range of activities in the Group. It is an established part of the Spectrometric Solution SRM program, discussed below. In addition, we have continued to promote its broader dissemination. The previous lithium aluminate work was documented for DoE and a publication is in review. The method is now being successfully applied by DoE to qualify material to the narrow specifications allowed for producing tritium safely in a civilian reactor. The ability to achieve high performance using two other manufacturers' ICP-OES instruments was also demonstrated this year. One of these instruments was in the laboratory of a provider of secondary standards. The experiments were done as part of a beta test of our proposed NTRM program for solution standards. The results of this collaborative work was presented at two conferences. In the international arena, we have collaborated with EMPA in Switzerland to characterize a series of elemental solutions that will be distributed as part of a CCQM key comparison (CCQM K-8). The relative standard errors of replication for the four elemental solutions prepared (Fe, Cu, Al, and Mg) were all better than 0.01 %.

The improved ICP-OES method has been presented in the context of "replacing classical analysis." It matches the precision and approaches the understanding of sources of error of classical analysis, especially for cases where the sample can be reduced to a simple dilute solution, as is done for the spectrometric solution SRMs. We have extended the concept this year by assaying the major elements in a more complicated sample, SRMs 1775/2175, a high temperature alloy with Ni, Mo, Cr, and Co as its major constituents. A multielement experimental plan and calibration strategy was applied to determine these elements. The relative standard uncertainties for replication were 0.02 %. Because of the rigor and labor intensity required, classical analysis is an expensive and disappearing capability in analytical chemistry. The results of this work were presented to a group of analysts in the metals industry in July. These analysts echoed a common theme whenever this work is presented—"how do I procure this capability for my laboratory?" We will continue to promote the process of technology transfer both by working with manufacturers and by demonstrating and publicizing the possibilities.

One of the research areas generally studied by the Group was the improvement of instrumental performance in the presence of drift as described in one of the technical highlights. One application was in glow discharge optical emission spectrometry (GD-OES). This work identified a source of drift in GD-OES and showed how it could be accounted for by applying an appropriate calibration strategy. Subsequently, the manufacturer has made hardware modifications that should alleviate the source of the drift—an example of how our research can have immediate impact. A second research activity in GD-OES was the study of the analytical benefits of alternative and mixed gases. This work was started through a collaboration with Clemson University and funding by NSF. Various figures of merit were assessed for glow discharges using pure Ne, Ar/Ne and Ar/He mixtures, and compared to Ar. Initial results indicate that there are only minor benefits for the extra effort expended, and this conclusion runs counter to published observations in this field. Finally, a major research effort was undertaken in the analysis of SRM 1775/2175, a high temperature alloy, this year, as part of the Division's exploration of new methods for certification of metals. The comparative results versus XRF and other methods, *e.g.* HP-ICP-OES described above, were instructive and showed that there is still a great deal to be learned about the technique.

Inductively-coupled plasma mass spectrometry is now the measurement workhorse for the group, continuing the trend begun in 1985 when the first instrument was procured. This year, we procured the fifth instrument for the group and installed it in the Division's laboratory in Charleston, South Carolina. The first inorganic analytical chemist to be stationed in Charleston was identified and he spent the year in Gaithersburg becoming familiar with the methods and procedures that used by the group. He moved to Charleston in August, and has overseen the completion of the inorganic facility, anchored by the ICP-MS, but also including a clean sample preparation area to allow trace element determinations. While at NIST he participated in the certification of Hg in several materials, and performed other Isotope Dilution-ICP-MS measurements. He also collaborated in a research project that investigated the coupling of a commercial cold vapor atomic absorption spectroscopy (CV-AAS) instrument with ICP-MS for mercury measurement. This method was used in the certification program for SRM 1641d Trace Mercury in Water and SRM 966 Heavy Metals in Blood. The key innovation was the ability to quantify the results using two distinct methods, including isotope dilution mass spectrometry.

Thermal ionization mass spectrometry is still the method of choice for high precision isotope ratio measurements and the Group has developed an isotope dilution mass spectrometry method for chlorine that addresses the need to quantify this gaseous non-metal in complex matrices. This work builds on a previously developed ID-TIMS method for chlorine in blood/serum that is not generally applicable to other materials; a new procedure for high precision chlorine isotope ratio measurement; and, a new chlorine extraction procedure. This work is an extension of a postdoctoral research project to measure and understand natural variability of chlorine isotopic abundance in geological materials. The determination of concentration by isotope dilution is a logical adjunct. The pyrohydrolysis technique for extraction of the chlorine is also being investigated for extraction of mercury, and direct coupling to an ICP-MS. European and U.S. environmental agencies recently have expressed concern about chlorine and mercury emissions in the burning of fossil fuels and proposed rules to monitor these emissions. These rules will require standards to benchmark measurement of these elements in the fossil fuels—critical measurements needed to understand and assess the magnitude of the problem. The certification of both elements in existing coal SRMs will serve this benchmarking need.

The Group took over the production and certification of the Single Element Spectrometric Solution SRM program at the beginning of the year, which included transfer of two scientists from the Gas Metrology and Classical Methods Group. The process by which these SRMs are certified was completely reengineered. A system for the production of weighed aliquots of primary standard solutions was implemented. These primary materials will serve as the basis for a new class of elemental solution SRMs designed for use as traceability transfer standards, and will be used in the development of an elemental solution NTRM program, described further in the NTRM Technical Activity Report 16.

Organic Analytical Methods

Research activities in organic analytical methods are directed toward the development, critical evaluation, and application of methods for the identification and measurement of organic and organometal species using organic mass spectrometry and analytical separations techniques. These separation techniques include gas chromatography (GC), liquid chromatography (LC), supercritical fluid chromatography (SFC) and extraction (SFE), capillary electrophoresis (CE), and capillary electrochromatography (CEC).

Recent research activities in organic mass spectrometry have focused on the development of techniques for characterization and quantitative determination of proteins in biological matrices. We are developing a number of primary and reference methods for important biomarkers such as troponin-I (a new marker of myocardial infarction), glycatedhemoglobin (an important new marker for diabetes), and serum thyroxine (an important marker for thyroid function). Techniques have been developed for the chemical characterization of purified troponin-I preparations, thus providing a means for comparing the molecular structure with results from field studies involving immunoassays. Two new LC/MS instruments are providing new capabilities for the determination of analytes of health, nutritional, forensic, and environmental importance, as well as for structural studies of natural products. Our new high-resolution mass spectrometer is being used in the development of methods for complex environmental contaminant mixtures such as dioxins, furans, and toxaphene as a prelude to future certification measurements of these substances in natural matrix SRMs. Ion-trap mass spectrometry is being combined with chemical ionization (CI) to improve the measurement of polyunsaturated fatty acids. Fatty acids such as these are found in fish oils and are believed to provide health benefits; however, their determination by mass spectrometry is hindered by their extensive fragmentation under electron ionization conditions, but with CI and acetonitrile as the source of reagent ions, it is possible to obtain strong signals for characteristic ions from these compounds. Mass calibration of mass spectrometers used for high molecular weight species is difficult and few appropriate materials are available. Research is underway to develop a set of proteins produced by bacteria grown in a media depleted in ^{13}C and ^{18}O. Such proteins will have much simpler isotope clusters which will be useful for mass calibration in matrix assisted laser desorption ionization (MALDI) and electrospray mass spectrometry of biomolecules.

Research activities in separation science continue to focus on investigations of the physical and chemical processes that influence retention in LC, GC, SFC, CE, and CEC. Results from these fundamental studies are used to design stationary phases tailored to solve specific separation and analysis problems, and to assist in method development and optimization. Recently we have explored a novel approach to the synthesis of LC stationary phases based on polymer immobilization. Polyethylene acrylic acid copolymers were immobilized on silica as an alternative to conventional silane surface modification chemistry. The resulting columns were evaluated for the LC separation of carotenoid isomers, and preliminary results indicate exceptional selectivity for this class of compounds. Further optimization of this promising stationary phase should result in improved measurement capability for carotenoids and related nutrients in food and tissue samples. Research in chiral separations is continuing in several areas using LC, CE, and GC. The determination of chiral drug species in hair samples using LC may permit environmental exposure to be distinguished from illicit use. Studies have been carried out with a model chiral compound (metoprolol) to evaluate the potential of this approach, for eventual application to amphetamine and methamphetamine. In other studies, functionalized cyclodextrins have been evaluated as chiral selectors in CE. The use of dimethyl-, hydroxypropyl-, and sulfated- forms of β-cyclodextrin improve aqueous solubility and alter the chiral recognition properties of this compound. Finally, chiral GC methods have been developed for the determination of DDT and DDE in environmental samples where enantiomeric resolution required coupling of chiral and achiral columns.

Insights gained from our research in separation mechanisms have provided us the knowledge to develop two new LC column performance test mixture SRMs. SRM 870, Column Performance Test Mixture, will be used to characterize LC column properties such as silica silanol activity, trace metal activity, and separation efficiency. SRM 877, Column Selectivity Test Mixture for Chiral Separations, will be useful in predicting the performance of columns for resolving enantomers.

Our research in organometal speciation has continued with improvements in the GC-atomic emission-based method for methylmercury and alkyl tin species. The new approach involves derivatization and a solid phase micro-extraction (SPME) step to concentrate the analytes and has been used to provide data for certification of methylmercury in SRM 2977 Mussel Tissue and SRM 1566b Oyster Tissue. During the coming year the method for methylmercury will be applied to additional SRMs including SRM 1946, Lake Superior Fish Tissue, and SRM 966, Toxic Metals in Blood. This same general approach will also be used to measure tributyltin in several of the marine matrix SRMs. Other research has involved development of multidimensional chromatographic approaches for quantification of non-ortho polychlorinated biphenyl congeners (considered to be the most toxic congeners) in some of our existing cod liver oil, sediment, and whale blubber SRMs, and nitro-substituted polycyclic aromatic hydrocarbons in diesel-related SRMs.

We have developed a method using pressurized fluid extraction and GC/MS or GC with electron capture detection to assess the micro-homogeneity of organic contaminants in natural environmental matrices. The method has been applied to air particulate, diesel particulate, frozen mussel tissue, and sediment SRMs. For the air particulate SRM, the amount of material that yields a 1% sampling error (due to heterogeneity) varies with analyte from approximately 50 mg to 450 mg, whereas the amount of diesel particulate material that yields a 1% sampling error varies only between 40 mg and

80 mg. Certificates of Analysis for these SRMs will be updated with this new information.

Even with the downtime experienced by the move to our new quarters, a number of high priority natural matrix SRMs were completed this past year. These were:

- **SRM 1546 Meat Homogenate**, which was identified by the USDA, FDA, and the food industry as a high priority need was completed with certified values for cholesterol, six fatty acids, calcium, sodium, iron, and total nitrogen, and reference values for proximates, calories, a number of other fatty acids, and some additional nutrients.
- **SRM 1944, New York/New Jersey Waterway Sediment**, with over certified values for 70 species including several polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated pesticides, and trace elements. Reference Values were provided for 80 additional species including selected polychlorinated dibenzo-p-dioxins(PCDDs) and dibenzofurans(PCDFs)
- **SRM 1649a, Urban Air Particulate Matter**, has certified/reference values for over 100 PAHs, PCBs, chlorinated pesticides, and PCDDs/PCDFs, as well as values for total organic carbon, percent extractable material, carbon composition, particle-size information, and trace elements.
- **SRM 1589a, PCBs in Serum**, has PCBs at naturally occurring levels in contrast to its predecessor where high levels of Aroclor mixtures were spiked into serum. This replacement material has been analyzed at NIST and the Center for Disease Control to provide certified and reference values for PCBs, chlorinated pesticides, and PCDDs/PCDFs.

A number of new SRMs are nearing completion. Details concerning these activities can be found Technical Activity Reports 12-14.

The research and standards development activities within this Group have resulted in over 50 manuscripts either submitted, in press, or published in refereed journals during the past year. Nine papers were published or accepted by the journal Analytical Chemistry, two of which were featured in the "A" pages, which are authoritative, high visibility reviews of specific topics written for a broad audience.

Gas Metrology and Classical Methods

Research activities within the Gas Metrology and Classical Methods Group are focused on gas metrology, classical wet chemical methods (gravimetry, titrimetry), coulometry, ion chromatography, and maintenance of the theoretical infrastructure for pH and conductivity measurements.

This year the move into the new building interrupted our programs for approximately three months. Despite this, we have had a very productive year due to the dedication and hard work of our staff. As seen below, this interruption had little effect on the number of SRMs, NTRMs, and Data Products delivered by this group. Also despite the move, the group was able to provide two courses, one of Classical Methods and the other on Gas Metrology, for representatives from other national standards laboratories within the Americas. These courses were very well received and are the only ones scheduled for repeat during the coming year.

During FY99 the following SRMs were completed; 15 gas cylinder, 3 anion solution, 12 conductivity, 2 ion activity, 6 Volatile Organic Compound solutions and a zeolite material. A total of 170 gas cylinder SRMs were recertified for 15 companies. While these certified reference materials are critically needed, their continuous production consumes considerable fiscal and human resources and limits our ability to address new standards needs. The gas NIST Traceable Reference Materials (NTRM) Program was created to address this concern. In FY99, we worked with 7 specialty gas vendors to certify 66 NTRM batches. The over 1000 individual gas cylinders in these certified batches will be used to produce approximately 100,000 NIST-traceable gas standards for end-users. Over the next year we will be defining a new approach for gas NTRM certification which will allow Specialty Gas Companies to expand their production while still maintaining the strong linkage to National Standards maintained at NIST.

Over the past year we have restocked all conductivity solution SRMs, thus recovering from a shutdown of this program two years ago when the person responsible for this program left NIST. In the past, leakage and transpiration limited our certification period to less than 1 year. Based on a pilot study involving three of our prime customers, we determined that switching to serum bottles provides stability for more than two years. We are planning on moving all conductivity solution SRMs into this bottling scheme in the next year. In addition we have revived a comparison with Hungary and Denmark on conductivity, and have championed a paper on molality-based electrolytic conductivity through IUPAC.

The area of pH standards continues to receive much international attention. Our active participation in IUPAC Commission V.5 resulted in acceptance of the Bates-Guggenheim convention, thus assuring continued traceability of pH to sound thermodynamic principals. We were also instrumental in defining the recommended values of uncertainty for the draft document on pH standards. This effort completes a three-year activity to defend the present pH scale from a simplified, yet non-traceable, scale championed by a few participants in V.5. Conversion to a new scale would have needlessly impacted U.S industry financially. Because of our active leadership in V.5, Ken Pratt has been nominated for Titular Membership in IUPAC. NIST also participated in both CCQM and European Union comparisons on pH. We have also organized an comparison to assess pH measurement capabilities within SIM.

Other activities in the classical methods program include research to improve the quality of our anion solution SRMs and certification of Si in a zeolite material using gravimetry coupled with XRF to account for additive interferences. This approach allowed the certification of Si in the Zeolite SRM with a relative uncertainty of 0.1 %.

In collaboration with EPA and the remote sensing community, we have developed a quantitative database of infrared spectra, which is required for establishing FTIR-based technology as a reliable tool for real-time monitoring for airborne chemical contaminants along plant boundaries and within plant facilities. Because the spectra are being prepared using NIST primary gas standards, well-defined traceability to NIST can be established for any subsequent field measurements. These spectra will be required to be used in the new update of EPA method TO-16. More information concerning this activity, and the most recent release of a CD-ROM containing spectra for 30 high priority species, can be found in Technical Activity Report 7. Future efforts will address continued data acquisition for compounds listed in the 1990 Clean Air Act amendment that are measurable with FTIR as well as additional compounds that contribute to global warming and ozone depletion.

In June 1999, the Ozone standards activities were transferred from the Surface and Microanalysis Division and integrated into the Gas Metrology program. NIST ozone standards effort supports a network of 22 Reference Photometers that provide NIST traceability for ozone measurements for the EPA and many International agencies. These Standard Reference Photometers (SRP) were designed and built at NIST and certified against the U.S. National Reference Photometer maintained at NIST. Currently we are upgrading these field photometers with new hardware and software

To assist the U.S. automobile industry, we are developing and evaluating new analytical measurement tools and providing standards needed to address compliance issues associated with the Clean Air Act of 1990. We are developing an infrared-based sensing device and are working with the NIST Physics Laboratory to evaluate a new Fourier Transform microwave device for real-time detection of oxygenated hydrocarbons in automobile exhaust. A series of new cylinder-based gas SRMs for low emission vehicles also are being produced to support U.S. automobile manufacturers in the development of the next generation of "clean" cars. For example, we are currently developing low concentration (<5 µmol/mol) nitric oxide gas standards to meet future regulations on automobile emissions. This effort will take approximately one additional year to complete and will result in new gas cylinder SRMs, since these standards are not suitable for the NTRM program. We will be collaborating with the Netherlands Measurement Institute and the National Physical Laboratory of the UK in this effort.

Molecular Spectrometry and Microfluidic Methods Group

The Molecular Spectrometry and Microfluidic Methods Group conducts research on or has responsibility for:

- the metrology of molecular spectrometry and develops standards for calibration, validation, and performance of instruments for measuring molecular spectra;
- conducts research on microfluidic devices, methods, and applications for chemical analysis including studies of materials and material properties affecting the flow of liquids in microchannels and the use of microchannel and other electrophoretic methods for forensic and toxicological applications and standards;
- the development and certification of optical transmittance and wavelength standards in the ultraviolet, visible, and near-infrared spectral regions; and
- works with users and manufacturers of analytical instruments to assess and measure the performance of analytical methods and to determine and address existing and future needs for analytical instrument standards ranging from device calibration and instrument performance through specifications for remote device control and data interchange.

In FY99, 102 solid absorbance filter SRMs were certified, and 189 optical filter sets were recertified. In addition, 250 units of SRM 2034 (holmium oxide uv-vis wavelength standard) and 98 units of SRM 2035 (NIR transmission wavelength standard) were certified. Measurements were completed on a 100 unit batch of SRM 2035a and research was conducted on a reflectance version of this material that will be designated SRM 2036. Development was begun on SRM 2037, a standard for calibrating the amounts of the red dye used to mark off-road diesel fuels for tax purposes, by acquiring a bulk material that contains the required Solvent Red 26 dye in ~ 85% purity. After further purification, the material will be certified for molar absorbance at two wavelengths in representative solvents. The new high accuracy reference uv-vis spectrophotometer was moved and installed in the ACSL. The software to control the instrument components was completed and validation and verification of the spectrophotometer has begun. A comparison of uv-vis absorbance filters (both gray glass on metal-on-fused-silica filters) was conducted between NPL, NIST-PL (vis only), and NIST-CSTL. All of the measurement results were with the uncertainty limits over the ranges that we support.

Three glasses (a chromium glass for 785 nm, a uranium glass for 532 nm, and an europium glass for 1064 nm) have been identified as promising candidate source intensity correction standards for Raman systems with 785 nm, 532 nm, or 1064 nm lasers. These glasses provide fluorescence spectra that, when calibrated against a standard white light integrating sphere source (calibrated by the NIST Optical Technology Division), can be used to correct Raman spectra for laser color and intensity variations. This in turn will allow Raman spectra to be made essentially instrument independent and will facilitate the development of Raman spectra libraries. At present, the chromium glass has been characterized sufficiently well to permit its being sent out to ASTM Raman subcommittee members for a round robin evaluation.

A program to develop standards for luminescence spectrometry was initiated with a Workshop at NIST attended by over 40 scientists including representatives from five National Metrology Institutes. Over 60 candidate substrates, glasses and fluor-impregnated PTFE materials, have been examined for their potential as fluorescence standards. In collaboration with the Biotechnology Division, we have begun developing a fluorescein solution SRM that will be certified for purity and for concentration. The material is to be used to peg the MESF (moles of equivalent soluble fluorophore) scale used in flow cytometry.

The optical filters NTRM program was announced publicly at a Pittcon Workshop in Orlando, FL and in two articles in Spectroscopy magazine. A NIST Special Publication (SP 260-140) and a supplement to NVLAP Handbook 150 that define this NTRM program were written, reviewed, and are now being sent to interested parties with an invitation to enroll in the initial class of NTRM producers. At least four companies are expected to participate and begin selling NTRM gray glass optical filters early in 2000.

We have initiated a project through the Office of Law Enforcement Standards at NIST to advance and support the concept of quantitative measurements for forensic analysis. Specifically, we have been developing a quantitative extraction and analysis method for the recovery of gunpowder additives based on ultrasonic liquid extraction and micellar capillary electrophoresis. In FY99, the technique was used to quantitatively compare the chemical composition of gunpowder residues to unfired gunpowder for identification. We are embarking on an effort to develop a standard reference material "Additives in Smokeless Gunpowder" that will help law enforcement agencies such as ATF and FBI to validate measurement methods for the composition of gunpowder and residue samples that are submitted for forensic identification.

We have partnered with the Electronics and Electrical Engineering Laboratory on a project supported through the NIST Advanced Technology Program to design and build silicon chips with microheating elements combined with channel interconnects in silicon, to devise methods for linking silicon chips to plastic microfluid devices, and to test heater operation in fluids for coupling to chemical reactions. We developed some new concepts to fabricate devices for this project and have leveraged some of the knowledge acquired in the course of this work to design new devices for a "Microscale Analytical Laboratory" project discussed in Technical Activity Report 6.

In conjunction with the Biotechnology Division, we provided the data analysis, analysis justification, value assignment, and certificate modification required for the recertification of SRM 2390, RFLP Profiling Standard. All forensic laboratories performing RFLP measurements are now required to analyze this SRM at least once a year to be eligible to receive Federal funding. Working with Guest Researchers from the Fochhochschule in Wiesbaden, Germany, we have extended our previous efforts to develop a device capability dataset (DCD) concept to the system level, defined and documented the system capability dataset (SCD), implemented a DCD for the HP 8453 spectrophotometer, and detailed the handling of result data by the SCD. Oliver Borchert received a second prize for his poster on dealing with result data using an SCD at the LabAutomation 99 conference in San Diego.

Nuclear Analytical Methods

Research activities in this group are focused on the science that supports the identification and quantitation of chemical species by nuclear analytical techniques. Current laboratory research activities involve the full suite of nuclear analytical techniques including instrumental and radiochemical neutron activation analysis (NAA), prompt gamma activation analysis (PGAA), and neutron depth profiling (NDP), as well as applications of neutron focusing technology for analytical applications. The measurement capabilities that reside within this group provide an excellent complement to those in the Spectrochemical Methods Group in that they depend upon characteristics of the nucleus of the element rather than the electron shells, and therefore are insensitive to chemical state. In addition, the nuclear analytical methods are generally nondestructive and thus do not require sample dissolution.

NAA is a powerful reference technique that has been used at NIST for many years. New developments continue to provide improvements in detection sensitivities, elemental specificity, precision, and overall accuracy that allow it to address new measurement needs. We have recently been developing Instrumental NAA procedures and applying them to study the homogeneity of SRMs at small sample sizes. Many analytical techniques used in industry and academia rely on the analysis of very small samples (*i.e.*, 1 mg), typically in the solid (undissolved) form. Unfortunately most SRMs are certified with minimum sample sizes of 100-500 mg, and are therefore unsuitable for use as control materials for these techniques unless additional information is made available. Taking advantage of the sensitivity and nondestructive properties of INAA, the use of this technique for homogeneity studies of small samples has been evaluated and implemented for the determination of sampling characteristics for a number of environmental SRMs. The minimal analytical uncertainty associated with the INAA measurements allows extraction of the variability due to material inhomogeneity from the observed total variability in a set of measurements. We have investigated three sediment materials that have shown the potential for a high degree of homogeneity at very small sample sizes for possible use as a future SRM aimed at solid-sampling analytical techniques. From the candidate materials a portion of the Baltimore Harbor Sediment, currently under certification for organic constituents (SRM 1941b) was selected for further processing as SRM for trace elements as well as for microanalytical techniques. Evaluation of the Ingamells model describing homogeneity has been initiated with the Statistical Engineering Division.

Instrumental neutron activation analysis has been used as a primary method for certification of the arsenic implanted dose in SRM 2134, Arsenic Implant in Silicon. This material has been a high-priority need of the semiconductor industry for a number of years. It was produced and characterized in collaboration with the Surface and Microanalysis Science Division and is intended for use as a calibrant for secondary ion mass spectrometry. The INAA results used for certification contains the first complete, quantitative evaluation of all sources of uncertainty in an INAA measurement. The expanded relative uncertainty for the mean value of this SRM was 0.38 % and approximates the 95 % level of confidence.

Significant advances have been made this year in the use of state-of-the-art signal processing techniques in our gamma-ray spectrometry systems that are used for INAA. Losses through pulse processing dead time and pile-up are best assayed with an external pulse technique. In this work, the virtual pulse generator technique as implemented commercially with the Westphal loss free counting module has been set up and tested with four high resolution gamma-ray spectrometers. Dual source calibration and decaying source techniques were used in the evaluation of the accuracy of the correction technique. Results demonstrated the reliability of the LFC with a standardized conventional pulse processing system. The accurate correction during high rate counting, including during rapid decay of short-lived activities, has become the basis for highly precise determinations in reference materials studies.

Pioneering research is conducted by this Group on the use of cold neutron beams as analytical probes for both PGAA and NDP. PGAA measures the total amount of an analyte present throughout a sample by the analysis of the prompt gamma-rays emitted immediately following neutron capture. NDP on the other hand, determines the concentrations of several important elements (isotopes) as a function of depth within the first few micrometers of the surface by energy analysis of the prompt charged particles emitted during neutron bombardment. Both of these techniques continue to make important contributions in the characterization of advanced materials, particularly with the enhanced sensitivities now available using cold neutrons from the NIST Reactor.

A method has been developed and an apparatus built to produce titanium (and other metal) SRMs of known hydrogen concentration on the few-kilogram scale. The method is based on the controlled reaction of hydrogen with titanium in a closed system. Homogeneity is checked by neutron incoherent scattering, and concentration is verified by cold-neutron prompt-gamma activation analysis and gravimetry. The first SRM material has been prepared near the critical level of 100 mg/kg. In addition, new materials will be prepared at two bracketing levels. The apparatus has also been used to prepare standards for neutron-tomographic nondestructive analysis of turbine blades at McClellan Air Force Base.

We are currently developing and critically evaluating a nondestructive technique to quantitatively determine hydrogen based upon neutron scattering. This will serve as a complement to PGAA measurements for this element. The incoherent scattering cross section of neutrons by the H nucleus is much greater than that of any other nuclei; and the H scattering cross section is much greater than the absorption cross section. These two properties accentuate the potential of neutron incoherent scattering (NIS) for rapid H determination. In addition, NIS has very-limited overlap of error sources with PGAA, and agreement of results between the two techniques provides a high degree of confidence.

We have begun measurements of nitrogen in chemical vapor deposited diamond films by NDP. Several problems have been identified including the elevated background from gamma-ray induced electrons. While the initial results are encouraging, more effort needs to be made to improve the signal-to-background levels. These improvements will be useful in future measurements of SiON, which is of importance to the next generation of semiconductors. We are continuing our measurements of lithium migration in thin films, as applied both to electrochromic multilayers and to lithium batteries. NDP is well suited to these studies as lithium mobility can be observed in "real" time.

The cold–neutron PGAA and NDP spectrometers, which are available to outside researchers as part of the NIST Center for Neutron Research "national users' facility", continue to provide high-quality measurements for outside scientists. Current experiments of interest at the NDP instrument include the measurement of shallow-doped boron content in silicon in conjunction with Advanced Micro Devices, calibration of boron in-house standards for Lucent Technologies, and the characterization of boron and nitrogen thin films in a wide variety of materials for high tech applications.

Non-destructive, matrix-independent measurements of hydrogen by the cold neutron PGAA technique have become particularly valuable in view of the difficulties of accurate determination of this element by other techniques. Many of the current PGAA experiments involve measuring hydrogen in a wide variety of materials for different applications. These include: metal embrittlement studies, studies of materials for new types of lithium batteries and fuel cells, and the determination of H concentrations for materials prior to neutron scattering measurements. PGAA has also been used along with neutron incoherent scattering (NIS) to map the hydrogen content of wet concrete samples as a function of position in an effort to create a moisture profile of the material and hence monitor the uniformity of drying. Other measurements made at the PGAA facility this year include: the determination of H, S, Ca, and K in Nafions, derivatives of Teflon which have potential use as membranes in electochemical separations and in fuel cells; the characterization of antiferromagnetic materials which are of interest to the superconductor industry; and the analysis of a Martian meteorite, to serve as a prototype spectrum in the design of a PGAA instrument for analysis of the Martian surface. PGAA has also been used with focused neutrons in the determination of chromium in a chromite layer deposited on steel casings that are used in Kraft recovery boilers by the pulp and paper industries for process chemical recovery.

Efforts continue within the group to develop the techniques and methodology of focusing cold neutron beams for analytical applications of PGAA and NDP. A monolithic lens consisting of a fused tapered bundle of polycapillaries that provides a smaller focus of about 0.15 mm (FWHM at the focal spot) has been installed in the NDP chamber. The chamber has also been modified to accept a separate lid through which the lens can be mounted for remote-controlled alignment. A new alignment system containing a CID camera with vacuum capability has also been installed. It provides a spatial resolution of 12 µm (pixel size) and an 8 bit dynamic range for the neutron intensity, and provides the possibility for charged particle imaging. Together with the new vacuum-compatible motion stages, the alignment system permits measurements without breaking the vacuum. Furthermore, the lens can be retracted out of the beam for measurements on the same sample with normal NDP geometry. The new lens and focusing system now gives us the capability of measuring samples as small as ~0.1 mm in diameter or, together with our current scanning system, of measuring concentrations in three dimensions with much improved spatial resolution. Previously, our three dimensional profiles were performed with beam spot sizes of 1 cm.

The Group organized and conducted the highly successful "Tenth International Conference on Modern Trends in Activation Analysis" in April 1999. This quadrennial meeting brings together the worldwide community to assess nuclear analytical techniques and their applications, with emphasis on innovative recent developments. This session was attended by 204 scientists from 37 countries.

Future Directions

All Division research and service projects are reviewed on an annual basis for quality, progress, and match to mission and customer needs. The chemical measurement science and cross-cutting program areas on which the Division's research and measurement service activities are focused will not change very much in the coming year. We will continue to redirect some of our efforts to expand our programs in analytical instrument calibration transfer and performance and clinical biomarker standards. As mentioned earlier, we are beginning a major new effort in microanalytical device measurement technology.

We will continue to focus more of the Division's resources on chemical measurement science and the development SRMs with improved accuracy and uncertainty levels, and expand our collaborations with commercial reference material suppliers for providing NIST- traceable secondary standards to meet day-to-day end- user needs. The Division's budget for Standard Reference Materials activities has been ~$3M for each of the past several years. During this period, the majority of our efforts have focused on renewals urgently requested by customers and few resources have remained for development of standards to address new measurement problems/needs. The gas NTRM program described previously has provided some relief and over the past three years, we have been able to initiate efforts for development of a number of new, high priority SRMs including the following that will completed during the coming year:

- **Aircraft Alloys** (P and S to be certified; materials failure related to P and S concentration)
- **Drinking/Wastewater Standards** (to provide accuracy benchmark and measurement traceability for proficiency testing of >5000 environmental laboratories conducted by commercial providers)
- **Fish Fillet** (PCBs, pesticides, methylmercury and food proximates to be certified)
- **Isotopically-depleted Protein Mass Standards** (with 831; for use in calibrating mass scale in 1,5000-50,000 dalton range)
- **Lubricating Oil Additives** (to certify 12 elements for providing QA and required measurement traceability for $5B/yr Lubricants Industry)
- **Moisture in Crude Oil** (moisture levels, like sulfur, greatly influence price of crude oils)
- **Transmission NIR Wavelength Standard** (For precise calibration of spectral region of increasing industrial interest)
- **Zeolite Catalysts** (Na, Si, Al, Fe, and trace metals to be certified)

In addition, work is continuing on multiyear projects with SRMs scheduled for completion in FY01 for the following:

- **Cardiac Troponin I** (new definitive biomarker of heart attack not being effectively utilized due to measurement problems)
- **NIR Transflectance** (wavelength standards suitable in form for calibration of single- and double-pass transmission in both wavenumber and wavelength)
- **Liquid Baking Chocolate** (to complement suite of SRMs developed in response to new nutritional labeling laws)
- **Frozen Spinach** (to complement suite of SRMs developed in response to new nutritional labeling laws)
- **Antibiotics in Milk** (for QA of HPLC tests used to accept/reject >50,000 lots of milk per year)

Work will begin during the coming year on several new SRMs for new clinical diagnostic markers:

- **Folates** (important antioxidants; neural tube defects and blood homocysteine levels)
- **Homocysteine** (marker for heart attack risk)
- **Glycated Hemoglobin** (diabetes diagnostic marker)
- **Ionized Calcium** (important marker for diagnosis of various disease states such as skeletal resorption and stone formation in the urinary tract)
- **Thyroid Stimulating Hormone** (marker of thyroid function)
- **Speciated Iron** (marker for anemia and hemochromatosis; to address clinical need to measure iron associated with various iron-containing proteins)
- **Bilirubin** (marker for liver function)
- **Total and Speciated Selenium** (to support clinical monitoring in the relatively narrow range of beneficial effect}

The major driver for this escalation of our clinical standards program is an *In Vitro* Diagnostic Devices Directive (IVDD) scheduled for initial implementation in December 2003. The directive requires all manufacturers of IVDD products sold in Europe to have an "EC Stamp", verifying that they comply with the conditions of the directive. U.S. companies are major exporters of IVDD products to European markets, and thus are directly affected by the directive. One of the major components of this directive is a requirement that products be traceable to "standards of the highest order", *e.g.*, nationally/internationally recognized certified reference materials (CRMs). At present, neither CRMs nor reference methods are available for most of the "A list" analytes that are well-defined chemical species of which there are approximately 80, and "B list" analytes that are less well-defined and number in the hundreds.

Additional SRM projects being initiated this year include the following:

- **Low Sulfur in Gasoline** (to support proposed U.S. regulation of sulfur content in gasoline for EPA Tier 2 Clean Air Act Amendments)
- **Low Nitric Oxide Standards** (to support measurements needed for development of very low emission vehicles and future automobile emissions regulations)
- **Anion Solutions** {nitrate, bromate, chlorate, chlorite, and cyanide)
- **Gravimetric Elemental Primary Standards** ("Mole standards for metals")

- **Very Volatile Organic Compounds in Methanol**
- **Organics in Household Dust** (for toxic organic contaminants such as pesticides and PAHs)
- **Fluorescein Fluorescence Intensity** (for calibration of instrumentation widely used by clinical and biotechnology communities)
- **Trans Fatty Acids in Foods** (to support proposed trans fatty acid measurements/nutritional labeling for foods)
- **Anode Tin** (to certify lead and material purity in this important steel coating material manufactured, used, and recycled worldwide; lead content of the recycled high-value tin is of particular importance for food safety, product liability, and environmental concerns)
- **Gunpowder Composition** (to validate forensic methods used to identify gunpowder residues)

Approximately 25 SRM renewals will be completed and efforts will begin for re-certification of an additional 15 or so high priority materials. For the longer term future, we plan to re-institute the NIST program in luminescence standards. The highest priority will be the development of SRMs for fluorescence measurements.

We expect that the NTRM approach will continue to expand as the basis for allowing the commercial sector to provide reference materials to end-users with a well-defined traceability linkage to NIST. Our Optical Filter Standards program will begin in January 2000 with our Elemental Standard Solutions and metal Alloys programs following about a year later. High quality SRMs will continue to be developed to address both national and international measurement problems, but they will be much more expensive and targeted for purchase primarily by other national metrology institutes and commercial producers/distributors. Increasingly, end-user needs will be met via NIST-traceable commercially produced reference materials.

The complex nature of chemical measurements coupled with the increasingly global nature of trade, health, and environmental issues speaks to the need for having the world make measurements using a common "meter stick". The comprehensive nature of the NIST program in chemical measurements puts us in position to make a very significant contribution to the international chemical measurements community in this regard. In order to do this in both a cost-effective and internationally congenial manner, we are investigating opportunities for partnering with other highly qualified NMIs to provide high quality Certified Reference Materials in specific standards areas. Potential advantages of these proposed partnerships include increased supply, breadth and quality of reference materials for the worldwide chemical measurements community as well as less duplication of effort for more efficient use of limited resources.

The information that has been provided represents a broad range of research and service activities within the general area of chemical measurement science. This overview plus the Selected Technical Activity Reports that follow provide additional details concerning our work and reflect the high quality of our staff. This information also shows how our efforts are impacting U.S. industry's productivity and competitiveness as well as providing measurement infrastructural support for environmental quality and human health assessments.

B. Selected Technical Reports

1. *Development of a Neutron Incoherent Scattering Method to Quantify Hydrogen in Metals*

H. Chen-Mayer, *R. M. Lindstrom, D.F.R. Mildner, and M. Blaauw (Interfaculty Research Institute, Delft, The Netherlands)*

Objective: To develop and critically evaluate Neutron Incoherent Scattering for the determination of hydrogen in metals.

Problem: It is well known that hydrogen causes embrittlement in metals. Methods used in industry for determining H in metals are highly matrix dependent. Therefore, the metals industry requires SRMs containing H in a variety of alloys to calibrate and verify laboratory methods. Although we have one method based on PGAA for non-destructive H determination, a second method is highly desirable for confirming analytical results. The new measurement method is based on the incoherent scattering of neutrons by the H nucleus, which differs from PGAA in that the latter relies on the absorption of a neutron and subsequent emission of a 2.2 MeV gamma ray by H.

Approach: The incoherent scattering cross section of neutrons by the H nucleus is much greater than both the scattering cross section of any other nucleus and the absorption cross section of H. These two properties of the neutron interaction with H establish the potential of using neutron incoherent scattering (NIS) for H determination with higher efficiency and better sensitivity than PGAA. Since measurements can be made in minutes, compared to hours for PGAA, the NIS technique can be used effectively to study homogeneity. PGAA, however, is much more selective and matrix independent. The two techniques have a very limited overlap of error sources, and therefore, agreement of results between the two techniques provides a high degree of confidence.

A good understanding of the neutron scattering mechanism is needed for each type of sample analyzed by NIS. The value of the scattering cross section depends on the binding state of H, *i.e.*, whether it behaves more like a free gas (scattering is in the forward direction), or is rigidly bound to the matrix (scattering is isotropic). We have built a test system to measure scattering in both forward and backward directions to determine which of these mechanisms is appropriate. Using standard addition techniques and making the assumption that H is rigidly bound in metals, we have obtained quantitative results that are in agreement with PGAA measurements.

Results and Future Plans: We have determined H concentrations by NIS measurements in a series of titanium alloys (45-350 µg-H/g-Ti), and compared the results with those obtained by PGAA. All values are in agreement with the exception of one sample at 300 µg/g. Further investigation is required to understand this discrepancy. We have studied H loading dynamics in a rare-earth window coating material used for reversible opacity control. The window opacity is altered by varying the hydrogen gas pressure which in turn affects the subsequent hydride formation within the material. When conditions are varying, a fast probe like NIS is indispensable. PGAA is not an appropriate technique for these measurements since it can only provide quantitative information when the system is in a steady state. We are currently testing a series of samples containing approximately 100 µg-H/g-Ti to be used as a future SRM. The NIS method's advantage of rapid determination allows scanning measurements of large metal sheets to obtain information on concentration uniformity. Currently, the experimental station is time-shared with other measurement applications. Each time the equipment is changed a complete background reduction effort and a new calibration is required. We intend to build a permanent chamber with neutron-black walls and a high precision sample-positioning device for the NIS measurement, with which the critical evaluation of the technique can be completed.

2. *Synthesis and Characterization of Novel Stationary Phases for Liquid Chromatography*

L.C. Sander, M. Pursch, and J. Wegman (Univ. of Tuebingen, Tuebingen, Germany)

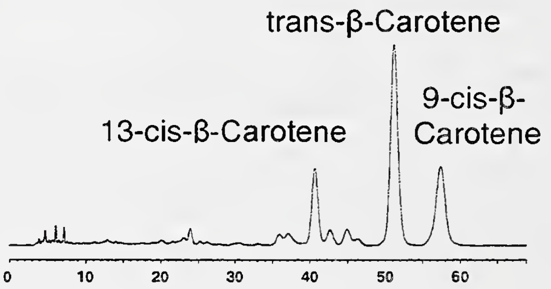
Objective: To enhance NIST capabilities for determination of isomeric species in natural-matrix samples through development of novel chromatographic stationary phases.

Problem: The liquid chromatographic (LC) separation of certain classes of isomers can present a significant challenge to the analyst. In addition to polycyclic aromatic hydrocarbons (PAHs), carotenoids and certain other nutrients are examples of compounds that occur in highly isomeric mixtures. The complexity of these mixtures is increased by other sample matrix elements, adding to the difficulty of analyses. Because the biological activity of contaminants and nutrients differ with molecular structure, measurement of individual isomers is required for assessment of potential adverse or beneficial health effects. Conventional commercial C_{18} columns do not exhibit sufficient inherent selectivity toward shape-constrained isomers to permit determination of many such species.

Approach: In recent years, research efforts within the Organic Analytical Methods Group have been directed toward improving analytical measurement capability for carotenoids, retinoids, tocopherols, and related nutrients in foods and blood serum. Part of this effort has involved the synthesis and characterization of novel LC stationary phases with enhanced selectivity toward carotenoid isomers. A highly successful result of this research has been the development of a column based on a polymeric C_{30} surface modification scheme. Further improvements in column performance are anticipated by the development of stationary phases based on longer alkyl chain lengths. Unfortunately, suitable reagents to prepare such long chain length stationary phases are not commercially available and cannot be synthesized in practical quantities or sufficient purity. To overcome these difficulties we have utilized a novel approach to surface modification involving immobilization of a poly(ethylene-co-acrylic acid) polymer by chemical and physical means.

Results and Future Plans: A series of stationary phases were prepared with poly(ethylene-co-acrylic acid) polymers with different composition. Three approaches to immobilization were studied: aminopropyl linkages, glycidoxypropyl linkages, and physical immobilization (*i.e.*, no chemical bond). Columns were prepared and characterized by use of SRM 869 "Column Selectivity Test Mixture," candidate SRM 870 "Column Performance Test Mixture," and various carotenoid isomer mixtures. Initial results are extremely encouraging. Columns exhibited high chromatographic efficiency with symmetric peak shape, and retention behavior was highly shape-selective. Most importantly, separation of several important carotenoid isomers was achieved with the new polyethylene columns (Cf. figure).

The results shown represent our first efforts using this new synthetic approach. Optimization of column performance will require further study of the reaction parameters, including polyethylene copolymer composition, reagent concentration, type of covalent linkage, choice of silica, and reaction time. It is anticipated that the improved selectivity exhibited by polyethylene stationary phases will help to eliminate measurement biases for carotenoids that result from component coelution.



Separation of carotenoid isomers with a polyethylene stationary phase.

3. *Maximum Likelihood Cosine Inversion for Dynamic Range Extension of Interferometric Spectra*

*M.L. Salit and **J.C. Travis***

Objective: To demonstrate the utility of optical interferometry in the UV and visible spectral regions for precise and accurate optical isotope ratio determinations; and to characterize "Maximum Likelihood Inversion" as an approach for obtaining spectra with physically meaningful noise distributions.

Problem: Fourier transform spectrometry has dominated grating spectrometry in the infrared spectral region for some years, due to the so-called multiplex advantage associated with acquiring data simultaneously at all available wavelengths. Technical advances in computing technology, control systems, and optical component fabrication over the past decade have made possible the successful operation of Michelson interferometers at shorter wavelengths – extending well into the UV – but without the multiplex advantage shown in the IR. Indeed, FT/UV is widely characterized as having a "multiplex disadvantage" resulting from the artificial redistribution of noise in the spectrum by the mathematical Fourier transform used to derive a spectrum from a Michelson-measured interferogram.

Approach: A recent study shows the promise of an alternative method for deriving the spectrum from an interferogram, distributing the noise at the frequencies where it belongs. By employing "Maximum Likelihood Inversion" [Bialkowski, Appl. Spectrosc. 52, 591 (1998)], we are working to achieve adequate accuracy and dynamic range for practical optical isotope ratio measurement. This program pivots on the implementation of an expectation-maximization (EM) implementation of Maximum Likelihood (ML) Inversion, enabling dynamic range enhancement of line emission spectra. The published algorithm has been implemented and tested with small data segments on a desktop computer. The algorithm, particularly scalable to multiple processors, has been ported to a parallel architecture. Preliminary experiments have been performed to validate the parallelization.

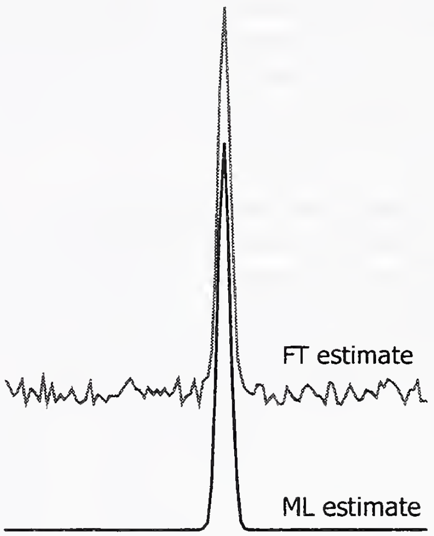
Because the computation of this inversion is costly, several strategies have been identified to effectively scale the approach to complete data sets.

Results and Future Plans: Scalability studies indicated excellent performance for the parallel implementation of the algorithm, with minimal loss in performance from inter-processor communication. The parallel implementation is portable between parallel computers, using standard libraries. Convergence performance was poor, and precluded inversions of even moderate resolution spectra.

Three technical approaches have been identified to achieve practical transform times with this iterative algorithm:

- region-of-interest identification and fitting
- filtered initial parameter estimation
- application of an accelerated EM algorithm.

Collaboration with scientists in the High-performance Computing and Statistical Engineering divisions has and will continue to be of utmost importance for further progress.



Maximum likelihood and Fourier transform estimate.

4. *Improvement of Instrumental Performance in the Presence of Drift*

M.L. Salit, S. Christopher, M. Epstein, G.C. Turk, and M.R. Winchester

Objective: To demonstrate the broader applicability of the Salit-Turk drift correction procedure for improving the precision of analytical measurements.

Problem: Low-frequency noise—often termed drift—results in a poor signal-to-noise ratio for repeated measurements of a given sample and is a common problem for analytical instruments. In 1998, Salit and Turk published an innovative strategy to overcome drift that used all the data in an experiment to create a model of the instrument drift response function. This new approach gave more than 10-fold improvements for precision in ICP-OES measurements. The authors speculated that the method would have general utility for precision chemical metrology and this speculation has now been verified in our laboratory using several analytical techniques, including glow discharge optical emission spectrometry (GD-OES) and atomic absorption spectrometry (AAS).

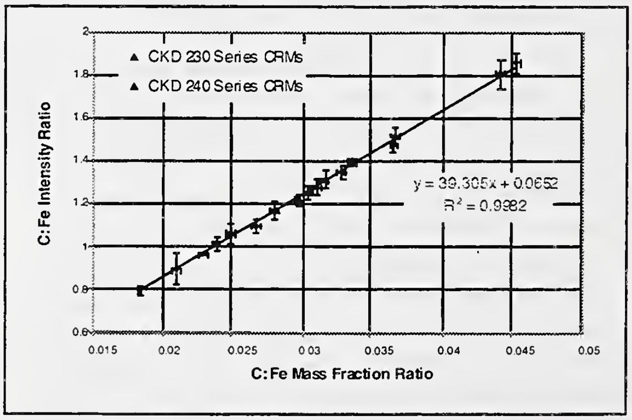
Approach: Drift correction methods that are usually used in GD-OES assume that calibration (and recalibration) can be done in a time frame that is short enough to be unaffected by drift. However, rigorous calibration in GD-OES—spanning a range of calibrants, repetitively measured—is time intensive. Drift does occur, compromising the validity of the calibration and possibly introducing analytical bias into the process. One type of drift that is observed in GD-OES is a burn-to-burn (sample-to-sample) variation of instrumental sensitivity and background. This drift can be caused by reduction of light transmission by the window that separates the glow discharge cell from the vacuum spectrometer.

The method's effectiveness for compensation of drift-induced calibration bias was tested by the determination of C, P, S, and Si in grey cast iron, using 17 CRMs as calibration samples. This analysis accentuated the problem—because of the time per sample and intense conditions required, the vacuum wavelengths measured, and the number of calibrants included.

The drift-corrected, carbon calibration data are graphed below. The well-defined calibration line illustrates the significant improvement that was achieved.

Experiments demonstrating the application of the Salit-Turk drift correction procedure in AAS, both flame and cold vapor, also have been done. In flame AAS, Cu, Fe, and Zn were determined in SRM 909 Human Serum using a dynamic dilution sample introduction pump system and internal normalization with Mn. Precision improved by factors between 3-6 for the elements measured. The procedure was applied in cold vapor AAS determination of Hg in Dust, SRM 2584. A drift of about 13% was observed over 5 hours of data collection, yet the relative precision for each sample ranged from 1-4% (1s). The Hg concentration determined in this material was 5.25 ppm (mg/kg) with a between sample relative standard deviation of 1.7%.

Results and Future Plans: The application of the drift correction methodology has improved the quality of NIST measurements, as noted above. The larger impact that can be achieved by its adoption and use outside NIST is also being promoted. Significant progress has been made in its transfer to the broader analytical community. Joint experiments have been done with both primary and secondary reference material providers in the U.S. and abroad; a successful collaboration has been completed with another agency of the U.S. government; and, several co-operative arrangements, both formal and informal, have been developed with analytical instrument manufacturers.



5. *New Analytical Methodologies for Environmentally Significant Organic Species*

M.M. Schantz, W.W. Brubaker, Jr., S.Tutschku, and S.A. Wise

Objective: To develop analytical methodologies for the quantification of methylmercury, organotins, and non-*ortho* polychlorinated biphenyls (PCBs) in natural matrices.

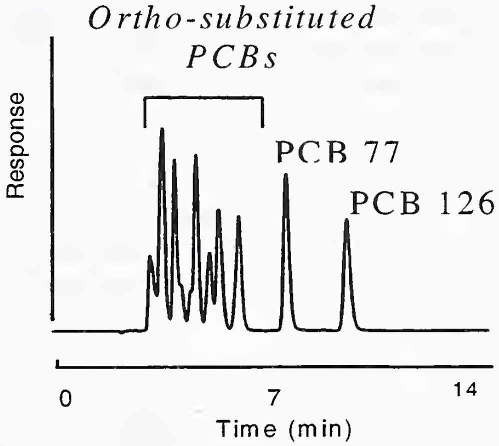
Problem: Analytical methodologies are well established for measuring *ortho*-substituted PCB congeners in natural matrix samples. The non-*ortho* PCB congeners, however, are potentially the most toxic and also generally the lowest in concentration. Because of coelution between the non-*ortho* PCB congeners, PCB 77, PCB 126, and PCB 169, and other chlorinated compounds found in natural matrix samples, an isolation step is needed to separate the non-*ortho* PCB congeners. Likewise, the speciation analysis of mercury and tin compounds has been of interest for some time since the organic forms are the most readily available to living organisms. The analysis of these species requires a selective extraction and analysis method.

Approach: New methods have been developed for measuring non-*ortho* PCBs and methylmercury in natural matrices and used to determine concentrations of the non-*ortho* PCBs in four SRMs: SRM 1588a, Organics in Cod Liver Oil, SRM 1945, Organics in Whale Blubber, SRM 1944, NY/NJ Waterway Sediment, and SRM 2974, Organics in Freeze-dried Mussel Tissue (*Mytilus edulis*). A known weight of sample was spiked with carbon-13 labeled analogues of the non-*ortho* PCBs and extracted and cleaned-up using the methods typically used for the analysis of these SRMs for PCBs. The additional step for isolation of the non-*ortho* PCBs from the *ortho*-PCBs is illustrated in the figure. Final analysis was done using gas chromatography/mass spectrometry with both electron impact and negative chemical ionization capabilities.

A new analytical procedure for methylmercury has been developed using microwave digestion in acidic media followed by phenylation and solid phase microextraction (SPME) with final analysis using gas chromatography with atomic emission detection (GC-AED). The use of SPME is critical as a PCB concentration step in the measurement of the low level of methylmercury in most natural matrices, and the AED is very sensitive and selective for mercury. Concentration of methyl-mercury in two tissue reference materials: SRM 2977 (Mussel Tissue-Organic Contaminants and Trace Elements) and SRM 1566b (Oyster Tissue) has been determined.

Results and Future Plans: Data for the non-*ortho* PCBs were compared to data available in the literature for these SRMs to validate the new analytical method. Results will be provided as reference values updating Certificates of Analysis. Methylmercury data from the SPME GC-AED method were compared to results from two other laboratories using analytical procedures different from the NIST procedure. All results were in excellent agreement, thereby validating both the new NIST procedure and the methods used by the other two laboratories to provide the literature data. These results have been statistically combined to assign a certified concentration for methylmercury in SRM 2977 (36.2 ± 1.7 ng/g dry mass basis) and SRM 1566b (13.2 ± 0.7 ng/g dry mass basis).

Methodology will be developed for dibenzo-*p*-dioxin and dibenzofuran congeners, as well as tin species, *e.g.*, tributyltin, in existing natural matrix environmental SRMs. The methods developed for the non-*ortho* PCBs and methylmercury will provide starting points to develop appropriate methods.



*A 2-(1-pyrenyl)ethyldimethyl-silylated silica (PYE) column chromatogram used to isolate the non-*ortho *PCBs demonstrating such a fractionation of 28 PCBs (excluding PCB 169) in* iso-*octane (SRM 2262). Hexane flowing at 1 mL/min was the eluent.*

6. *The Microanalytical Laboratory*

L.E. Locascio, M. Branham, W.A. MacCrehan, and M.L. Gaitan (812); and J. Xu and C. S. Lee (Univ. of Maryland)

Objective: To develop methods for monitoring and characterizing microchannels and microfluid flow.

Problem: The concept of the microanalytical laboratory continued to grow rapidly in the last year with more than one new product on the market and many more on the way that integrate microfluid components. The majority of new products are being fabricated in quartz substrates since much of the necessary research has been conducted in this material allowing for a shorter laboratory-to-market time. There is much less known about plastic materials in terms of fabrication and micro-channel characterization. In the first year of our competence effort, we have focused on understanding and characterizing flow and surface chemistry in plastic channels fabricated by NIST imprinting methods.

Approach: The process used to fabricate plastic microfluid devices can influence the surface charge on the microchannel wall as has been demonstrated for laser ablated channels. The surface charge and charge density on the channel wall are critical issues in microfluidics since both the rate and direction of electro-osmotic flow are a function of wall charge. Wall charge also controls the adsorption of chemical and biochemical species. We have previously characterized the surface change associated with our plastic microchannel devices by monitoring the electro-osmotic bulk flow using the current monitoring technique. This method has been used to successfully evaluate flow in plastic microchannels. However, it provides no direct information on the location and density of surface charge in fluid channels. In the past year, we have developed methods to probe the surface of imprinted channels with fluorescent chemical labels that enable the identification of specific chemical groups. We refer to this process as chemical mapping. With chemical mapping, we can determine the effect of our imprinting procedure on the channel charge and can alter our fabrication protocols to modulate this charge. Another drawback associated with the current monitoring technique is that flow measurement is not performed continuously. Because of this limitation, the current monitoring technique cannot be used to detect changes in flow during the course of an experiment that may be caused by analyte adsorption or fluctuation in the source pump rate. To address this problem, we have been developing methods for integrating silicon elements for flow monitoring and control within our plastic microfluid devices.

Results and Future Plans: Labeling of imprinted PMMA micro-channels with group-specific fluorescent probes indicated that mechanical stress induced the formation of carboxylate moieties that were concentrated in the channel walls and in surface defects. Non-imprinted materials and the channel floors did not reveal significant amounts of carboxylate or amine functional groups. Proteins were preferentially adsorbed to the negatively charged channel walls as compared to the floor as shown in the figure. The formation of carboxyate functionalities in the PMMA using the imprinting techniques will likely alter the adsorptive and electrto-omotic properties of the polymer microchannels. Integration of silicon components with plastic microfluid channels for flow monitoring has also been a focus of our research. Microheating elements fabricated in silicon have been successfully coupled to polymer channels. Preliminary results show that these devices can be used to continuously monitor fluid flow in integrated microfluid devices. Future work will focus on chemically modifying the microchannel walls to alter channel charge for fluid control for flow stability. We will also attempt to implement microheaters in microfluid systems for flow control with feedback.

Fluorescence Image of Microfluid Channel with Carboxylate-specific Probes.

7. *New Infrared Spectral Database Introduced to Support Remote Sensing Applications*

P.M. Chu*, F.R. Guenther, P.A. Johnson,*
G.C. Rhoderick, and W.J. Lafferty (844)

Objective: To develop a "Quantitative Infrared Database" to support open-path Fourier transform (FT) infrared measurements of chemical emissions and hazardous air pollutant (HAP) molecules.

Problem: Over the last decade, growing concerns about the environment in general and air quality in particular have stimulated the development of improved, cost-effective field monitoring methods. With FT infrared-based technologies multiple airborne chemical contaminants can be measured simultaneously – because each molecular species has a unique infrared spectrum–at part-per-billion level sensitivities. The *in-situ* and real-time nature of this approach offers several advantages over traditional point source monitoring techniques for applications such as determining fugitive emissions and chemical contaminants from industrial processing plants, hazardous waste and municipal landfills, water treatment plants, oil refineries, and chemical plants. Following successful testing of FT infrared methods during remediation of several Superfund sites, the U.S. Environmental Protection Agency (EPA) has issued a protocol (TO-16) for FT infrared open-path remote sensing. Successful implementation of this protocol is highly dependent on the availability of high-quality reference spectral data from a definitive source since molar absorptivity data in the literature widely differ.

A validated quantitative database traceable to national measurement standards is a critical part of the infrastructure required for establishing emerging infrared-based monitoring technologies. New infrared-based technologies coupled with the NIST spectral database provides both industry and EPA with a tool for assessing regulatory compliance that is both cost effective and less invasive.

Approach: SRD 79 data are based on NIST primary gravimetric standards prepared with starting materials of assessed purity and procedures that minimize contamination. For each compound, the absorption coefficient spectrum was calculated using nine transmittance spectra and the Beer's law relationship. The uncertainties in the absorption coefficient data were estimated from the linear regressions of the data and considerations of other error sources such as the non-linear detector response. For absorption coefficient values greater than 1×10^{-4} (μmol/mol) $^{-1}$ m^{-1}, the average relative uncertainty is 2.2 %. Comparisons with a number of expert laboratories, including the National Physical Laboratory of the UK, were used to assure the quality of the NIST data.

Results and Future Plans: Absorption coefficient data for 21 high priority HAPs on a U.S. EPA priority list has been published as the NIST Standard Reference Database (SRD) 79, version 1.00. Data for ten additional compounds will be available shortly. The data are stored in the standard JCAMP-DX format to enable universal access to the data. The 0.12 cm^{-1} resolution data were processed to generate data at a number of different resolutions and apodizations to provide users with data that closely match their experimental parameters. A digital signature accompanies each data file, allowing users to ensure the integrity and source of the data file and traceability to NIST.

This quantitative infrared database is an ongoing project at NIST. Additional spectra will be added to the database as they are acquired and updates will be available over the Internet. Plans include continued data acquisition for the compounds listed in the 1990 U.S. EPA Clean Air Act Amendment, as well as for those compounds that are of concern in global warming and emissions trading. Additionally, inter-comparisons of NIST primary standards and molar absorptivity data with National Metrology Institutes will be expanded to facilitate the use of this database in issues of global interest and impact.

Publications:

Chu, P.M., Guenther, F.R., Rhoderick, G.C., Lafferty, W.J., and Phillips, W.J., "***Sample and Data Processing Considerations for the NIST Quantitative Infrared Database,***" Proc. SPIE, 3534, 204 (1998).

Chu, P.M., Guenther, F.R., Rhoderick, G.C., and Lafferty, W.J., ***"The NIST Quantitative Infrared Database,"*** J. Res. Natl. Inst. Stand. Technol., 104, 59 (1999).

8. *A Henry's Law Constants Database for Environmentally Significant Compounds*

D.L. Poster, M.M. Schantz, H.A. Bamford, and J E. Baker (Univ. of Maryland)

Objective: To review critically and measure Henry's Law constants for environmentally significant compounds.

Problem: Physical property data for many hazardous organic contaminants listed in the U.S. EPA's Clean Air Act Amendment of 1990 are required by a wide range of communities, including those involved with (a) implementing environmental clean-up programs, (b) modeling the fate and distribution of organic compounds in the environment, and (c) assessing human health and environmental effects associated with exposure to pollutants. However, physical property data of organic contaminants are lacking or largely inadequate for estimating their environmental behavior. Large portions of physical constant data are suspect because reported values were made at lower levels of sophistication than available today.

Approach: A physical property database for environmentally significant compounds will consist of data obtained from the literature and from measurements. The literature review will collect references that contain information on physical constants such as surface area, heat of fusion, entropy of fusion, aqueous solubility, vapor pressure, and Henry's Law constant, and will document the measurement techniques used. One hundred twenty two references are reviewed for a wide range of compounds (Table 1). Reported values and the methods used to derive these values are extracted. Only references that contained data resulting from direct measurements are tabulated. Papers that replicate data from primary sources, are listed in the bibliography of the review. In the data compilation, each compound's Chemical Abstract Service number, molecular weight, physical constant value, method of measurement, and temperature of measurement are listed. Laboratory measurements were conducted to assess data for a range of PAHs and PCBs. Henry's Law constants were measured with equilibrium methods using assays with sufficient sensitivity for infinitesimally dilute solutions of contaminants. Simultaneous measurements of gaseous and dissolved concentrations near those found in the environment were made. Investigations of the temperature dependence of Henry's Law constants quantified temperature effects on the air-water distribution of 13 PAHs and 24 PCBs. The temperature dependence of the Henry's law constant for each compound is modeled using the van't Hoff equation to calculate the enthalpy and entropy of volatilization. These data can be used to extrapolate the Henry's law constants within the experimental temperature range, a unique part of this work. The experimental temperature range chosen represents temperatures within environmental systems.

Table 1. Physical Property Database Compounds

Compound Class	Number of Compounds
Polycyclic aromatic hydrocarbons (PAHs)	33
Chlorinated aliphatic compounds	19
Polychlorinated biphenyl congeners (PCBs)	152
Chlorinated benzenes	13
Polychlorinated dinbenzo-pdioxins	16
Polychlorinated dibenzofurans	56

Results and Future Plans: This physical property database contains 289 organic contaminant compounds. Henry's law constants for 13 PAHs and 26 PCBs have been experimentally determined between 4°C and 31°C. These data represent the first experimentally measured temperature dependence of the Henry's law constant for 9 of the 13 PAHs and 24 of the 26 PCBs. After using relationships between temperature and the Henry's law constants for interpreting the thermodynamic relationship between the enthalpy (ΔH) and entropy (ΔS) of phase change for these compounds, this program will be concluded.

Publications:

Bamford, H.A., Poster, D.L., Baker, J.E., "***Method for measuring the temperature dependence of the Henry's law constant of selected polycyclic aromatic hydrocarbons***," Polycyclic Aromat. Compd. in press.

Bamford, H.A., Poster, D.L., Baker, J.E., "***Temperature dependence of the Henry's Law Constants of Thirteen Polycyclic Aromatic Hydrocarbons Between 4 °C and 31 °C,***" Environ. Toxicol. Chem., 18, 1905 (1999).

Bamford, H.A., Baker, J.E., Poster, D.L., "***Review of methods and measurements of selected hydrophobic organic contaminant aqueous solubilities, vapor pressures, and air-water partition coefficients,***" NIST Special Publication 928.

9. *An Update to NRLXRF and NBSGSC – Fundamental Parameters in X-Ray Fluorescence Spectrometry*

J.R. Sieber and W.T. Elam (Naval Research Laboratory)

Objective: To bring up to date the landmark X-ray fluorescence (XRF) fundamental parameters computer programs NRLXRF (1978) and NBSGSC (1985). To update atomic parameters published since 1985 and to include an improved calculation of the spectrum from an X-ray tube. To publish this new database, to allow its critical evaluation and use.

Problem: XRF analysts apply fundamental parameter (FP) software to improve accuracy when they have few calibration standards. The programs NRLXRF and NBSGSC have served this purpose for over 15 years and still are requested, even though their algorithms, data files, and programming codes are significantly dated, limiting important applications, especially for custom spectrometer development. Commercial software developers have not adopted a standardized database form for the fundamental parameters and do not provide source codes that can easily be modified for research.

Approach: The fundamental parameters method relies on the use of equations that express the intensity of X-ray emission in terms of parameters that are independent of a particular sample. These parameters fall into two categories: instrument parameters and atomic parameters. Instrument parameters can be measured or are known *a priori*. The remaining parameters are associated with the elements of which the sample is composed and describe the X-ray properties of these elements. The properties of the elements together with the elemental composition of the sample can be used to compute the expected X-ray emission of the sample. The computations can be iterated to determine the composition of an unknown sample. The accuracy of the atomic parameters is crucial to the accuracy of the FP method. In particular, X-ray absorption cross-sections contribute directly to the quality of the results. Other parameters, *e.g.*, Coster-Kronig transition probabilities, produce smaller effects and high accuracy is not required. However, the more accurate the parameters, the more useful the calculations will be and the greater the accuracy that can be achieved.

Table 1. Fundamental parameters required for calculations in XRF. Parameters are needed for each element in a sample

Atomic number	Photoabsorption cross-section
Atomic weight	Coherent scattering cross-section
Pure element density	Incoherent scattering cross-section
X-ray absorption edges: Energy Jump ratio Fluorescence yield	Coster-Kronig transition probability: X-ray emission lines Energy Relative intensity

The authors undertook to produce a consistent set of parameters optimized for FP calculations using values available in the literature. The required parameters are given in Table 1. The newer and more complete IUPAC notation is used for X-ray emission lines. The calculation of spectra from X-ray tubes was based on recent work in electron microprobe analysis. The fast and versatile FP algorithm from NRLXRF was used because it handles mixtures of compounds and particulate samples rather than simple elemental compositions and it can be extended to multilayer samples by using the analytical solutions to Sherman's equations derived by De Boer.

Results and Future Plans: The results of this work, in the form of both a database and computer source code, will be made available to the public and to commercial XRF software developers. At present, the computer codes are nearly ready to perform the first FP calculations of intensity from samples to verify the output of the code and check for improvements in accuracy with the new parameters. Modifications for particulate and multilayer samples are planned. Finally, a graphical user interface will be added to the program as time permits.

10. *Real-Time Characterization of Lithium Transport in Thin Films*

G.P. Lamaze and H. Chen-Mayer

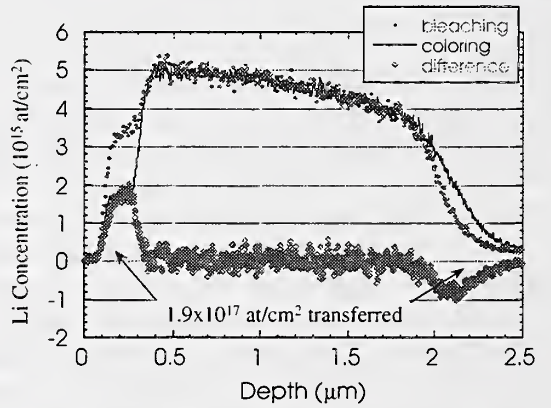
Objective: To measure lithium concentration and mobility in thin multilayer films in real time.

Problem: Lightweight, less toxic lithium-based batteries now dominate the market for laptop computer, cell phone and other consumer electronics. Market demand for all types of rechargeable cells is expected to grow at a compound rate of 11 per cent per year, reaching a total of 2 billion cells by 2001 of which the lithium ion cell share is expected to be 440 million cells. Thus the market for lithium ion cells will soon reach about $4 billion. In spite of this rapid commercialization, much of the underlying materials science is not completely understood. In these systems, a single ion species is shuttled back and forth between anode and cathode on charge and discharge. NDP can be used to examine the lithium distribution in anode and cathode materials to try to explain such problems as charge capacity loss with cycling, lower than theoretically attainable reversible charge capacity, and less than theoretically attainable maximum charging and discharging current densities.

Approach: First studies were undertaken with lithium electrochromic devices, which are similar to lithium batteries in both construction and operation. Samples are placed in a cold neutron beam at the Center for Neutron Research reactor. The lithium profiles are deduced based on the measurement of the energy of alpha particles from the $^{6}\mathrm{Li}(n,\alpha)^{3}\mathrm{H}$ reaction. The alpha particles pass through the layers and lose energy through interactions with the matrix. The residual energy of the particles is measured with surface barrier detectors. Because the starting energy of the alpha particles is fixed, the energy loss is obtained by a simple subtraction. This measured energy loss, when combined with a calculated energy loss per unit depth (dE/dx), provides the depth of the originating lithium nucleus. In this case, *in situ* measurements are taken with different bias voltages on the film layers. The bias causes the lithium to migrate between different layers while changing the optical transparency of the film. The profiling technique is non-destructive allowing repeated cycling of the device. The real time nature of the NDP measurement process allows us to watch changes in transparency as the lithium migrates between layers.

Results and Future Plans: The figure shows a typical profile obtained for lithium profile measurements of multilayer films. The small circles represent the device in its bleached state and show that most of the lithium is concentrated in the counter electrode (CE) and the ion conductor (IC). The small diamonds represent the device in the coloring state. In the colored state, the profile shows that lithium is primarily localized in the IC and the WO_3 though some lithium remains "bonded" in the CE. Among the information learned in this study was critical process chemistry, migration of lithium into inactive layers, and lithium loss from active layers during accelerated life tests.

We have begun the application of this technique to the study of another lithium single-ion conductor systems, *i.e.*, "rocking chair" batteries. With Tufts University, we have measured lithium content and lithium/cobalt ratios in $LiCoO_2$, a material that can be used as an electrode material in lithium batteries. The lithium content is measured by neutron depth profiling while the cobalt content is measured by neutron activation analysis. We plan to continue our collaboration with Tufts and expand our work in the area of battery technology.



Lithium concentration in two different states and the difference spectrum.

11. *SRMs to Support Chemical Measurements in Industrial Applications*

H. Chen-Mayer, S.J. Choquette, W.R. Kelly,
R.M. Lindstron, E.A. Mackey, J. Mann,
S.A. Margolis, A.F. Marlow, L. O'Neal, P.L. Paul,
J.R. Sieber, R.D. Vocke Jr., and L.J. Wood

Objective: To promote U.S. economic growth by working with industry to develop and apply measurements and standards.

Problem: The development of new technology and products, coupled with the evolution and advancements of analytical instrumentation used by industry, results in an ever-expanding need for standards. The Division balances its broad program in compositional and chemical standards among continuously produced standards (*e.g.* primary materials), renewals (previously certified materials), and new standards. The Division works with broad-based industrial groups to identify and produce new standards—which typically will require the development of new analytical methodology or refinement of existing methodology—research in analytical methods.

Approach: New standard activities reside in all functional areas and Groups of the Division. Each project has its own unique characteristics, but each also has common stages: an indication of need by some industry advocacy group; the development and definition of NIST capabilities; and, then, the application of these NIST capabilities in value assignment of a material available for broad distribution as an SRM.

Results and Future Plans: Five examples of ongoing projects are provided to illustrate the breadth of industrial measurement problems being addressed by the Division.

<u>SRM 2035/2035a Near Infrared Wavelength:</u> NIR spectroscopy is a technique widely used in process control for a variety of industries. Through interactions with two committees of ASTM the need for optical filter wavelength standards in this wavelength region was established. In addition, technical workshops and meetings were held with users and regulators to refine these needs. Several prototype materials were evaluated; a round robin to potential end users in the chemical, polymer, pharmaceutical, and analytical instrumentation industries were completed. Technical issues such as homogeneity, temperature and environmental stability, polarization and bandwidth effects required investigation. The first material has now been certified.

<u>SRM 861, Aerospace Superalloy:</u> The U.S. aerospace industry is a leading technology driver in R&D spending and the nation's leading net exporter of manufactured products. The aircraft industry and supporting foundries and their Consortium on Casting of Aerospace Alloys brought to NIST the need of the industry to produce turbine blades with sulfur concentrations near 1 mg/kg, a critical level between acceptable and unacceptable materials. Presently, the two established techniques used by industry to measure sulfur are discordant and both require standards for calibration and validation—standards that did not exist. The Division has investigated the extension of its primary isotope dilution mass spectrometric method to the low concentration required, accounting for the variability of the blank, the limiting source of uncertainty in this measurement. A material has been prepared to benchmark the need and NIST's measurement capability. Preliminary results indicate that the certified sulfur value will be an order of magnitude lower than any other standard.

<u>SRM 2721/2722 Moisture in Crude Oil:</u> The measurement of moisture in crude oils is important for the oil industry because of the large amount of crude oil that is recovered by a steam process and shipped saturated with water. ASTM methods existed for this measurement; however reference materials do not exist to benchmark the accuracy of these methods, or to gauge their robustness across materials and conditions. In preparation for certification, these methods have been critically assessed and sources of systematic bias have been enumerated. One result was the identification of a large negative bias for the recommended ASTM method D1533 if the oil is not completely dissolved or if the instrument is not properly calibrated. The development of a reliable method has resulted in moisture certification in two different crude oil materials at approximately 926 and 96 mg/kg. It is also intended to certify these two materials for their sulfur content, another chemical component that strongly affects crude oil value.

<u>Hydrogen in Titanium Alloys:</u> Hydrogen is one of the chief contributors to brittleness in metals; its measurement and control in certain alloys is critical,

especially for the aerospace and nuclear power industries. The principal method used in industry to measure hydrogen is calibrated with working standard materials. At one time NIST used this method and certified materials in co-operation with industry. However, NIST no longer supports either this technique or the mode of certification where no NIST measurements are included. The ASTM metals standards committee has steadfastly maintained the importance of benchmarking this measurement. Thus, a method has been developed that can produce metal standards of absolutely known hydrogen concentration. This method is based on the controlled reaction of hydrogen with titanium. Homogeneity is checked by neutron incoherent scattering and concentration is verified by cold-neutron prompt-gamma activation analysis. The first material has been prepared near the critical level of 100 mg/kg. In addition, new materials will be prepared at two bracketing levels.

SRM 1848 Lubricant Additive Package: The lubricants industry is estimated to be worth $5 billion in the United States. Although ASTM maintains a range of analytical methods for lubricants, none of the methods have bias statements, and there are no certified reference materials to verify the accuracy of analysis. Thus, a SRM certified for additive elements in passenger car motor oil was proposed to fill the need for traceable standards for this industry, and this standard is in the process of being certified in co-operation with industry laboratories. The candidate material was donated by one of the major U.S. producers, its homogeneity checked at NIST, and a round robin of major industry laboratories has been conducted. Quantitative procedures for XRF analysis have been implemented at NIST to benchmark established NIST and industry instrumental methods. At NIST, certification measurements for 8 elements, B, N, Mg, P, S, Cl, Ca, and Zn, by nuclear methods, XRF, atomic spectroscopy, and mass spectrometry are in progress. The goal is to achieve uncertainties for the NIST certified values of 0.5-1.0% to meet the industry's need (~3%).

12. *SRM Activities to Support Nutritional Labeling*

K.E. Sharpless, J. Brown Thomas, S.A. Margolis, C.S. Phinney, L.C. Sander, L.T. Sniegoski, L.K. Walton, and L.J. Wood

Objective: To develop food-matrix Standard Reference Materials (SRMs) to support nutrition labeling issues.

Problem: The Nutrition Labeling and Education Act (Public Law 96-359) requires that specific nutritional information be provided on all processed foods sold in the U.S. In a recent study by the U.S. Food and Drug Administration, the accuracy of label information ranged from 98% to 54% for nutrients for which labeling is required. As more food-matrix SRMs become available, label accuracy should improve when the food and nutrition communities employ these food-matrix SRMs in their analyses. Food-matrix SRMs are needed to validate analytical methods and for quality assurance when assigning concentration values to in-house control materials. Such reference materials will facilitate compliance with new nutritional labeling laws, provide traceability for food exports needed for acceptance in many foreign markets, and improve the accuracy of nutrition information that is provided to assist consumers in making sound dietary choices.

Approach: AOAC International has developed a nine-sectored triangle in which foods are positioned based on their fat, protein, and carbohydrate content. AOAC's belief is that one or two reference materials within each sector should be representative of other foods within that sector and could be used for quality assurance and method validation when analyzing those other foods. NIST does not have the resources or analytical capabilities necessary to measure all of the analytes for which labeling is required; therefore, nutrient concentrations in SRMs and RMs have been determined through collaborations with the food industry and food-related government regulatory agencies. Certified values are provided for analytes for which NIST alone (using two independent methods) or NIST and collaborating laboratories provide data. Reference or information values are provided for analytes for which only collaborating laboratories provide data.

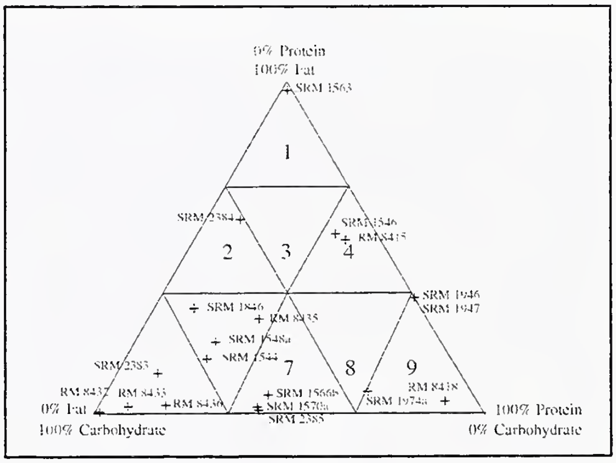
Results and Future Plans: NIST is actively working to provide an increased array of SRMs with values assigned for proximates (procedurally defined values for fat, protein, carbohydrate, etc.), fatty acids, cholesterol, vitamins, elements of nutritional interest, etc. SRMs and RMs are now available or are in preparation for the sectors in the AOAC triangle shown.

In 1998, NIST certified SRM 1546 Meat Homogenate, a canned meat product containing ham, pork, and chicken. This material was developed at the request of U.S. Department of Agriculture's Food Safety Inspection Service. Concentration values were assigned based on values from NIST, from an interlaboratory comparison exercise involving 17 member laboratories of the National Food Processors Association's (NFPA's) Food Industry Analytical Chemists Subcommittee (FIACS), and from several additional collaborating laboratories. Concentration values have been assigned for proximates, cholesterol, individual fatty acids, water-soluble vitamins, and elements of nutritional interest.

Using data provided by collaborating laboratories, values have been assigned for proximate concentrations in ten existing SRMs and RMs: SRM 1563 Cholesterol and Fat-Soluble Vitamins in Coconut Oil (Natural and Fortified), SRM 1566b Oyster Tissue, SRM 1570a Spinach Leaves, SRM 1974a Organics in Mussel Tissue (*Mytilus edulis*), RM 8415 Whole Egg Powder, RM 8418 Wheat Gluten, RM 8432 Corn Starch, RM 8433 Corn Bran, RM 8435 Whole Milk Powder, and RM 8436 Durum Wheat Flour. (Most of the materials in this group previously had values assigned only for concentrations of inorganic analytes of nutritional or toxicological interest.)

SRM 2384 Baking Chocolate and candidate SRM 2385 Spinach are currently in preparation. These materials were developed as a result of a workshop held at NIST in 1997 in which SRM needs of the food industry were identified. Materials in sectors 2 and 7 of the AOAC triangle and a vegetable material with values assigned for persistent pesticides were the top three priorities identified. SRM 2384 Baking Chocolate lies in sector 2 of the AOAC triangle; with the assistance of NFPA's FIACS, values will be assigned for proximates, individual fatty acids, total dietary fiber, caffeine, theobromine, several water-soluble vitamins, and elements of nutritional interest. Candidate SRM 2385 Spinach lies in sector 7 of the AOAC triangle. (This material will be a canned material, unlike SRM 1570a, which is freeze-dried and finely-ground spinach leaves.) NIST-specified pesticides will be applied to candidate SRM 2385 at normal application rates when it is grown. The material is expected to have values assigned for proximates, individual fatty acids, total dietary fiber, elements of nutritional interest, vitamins, carotenoids, folates, and pesticides.

Recently we held a food-related reference materials needs assessment workshop at the AOAC meeting in Houston, TX with broad representation from the food, nutrition, and regulatory communities. High priority needs identified at the workshop include SRMs for nutraceuticals, allergens such as peanut proteins, additives and preservatives, juice authenticity, chondroitin sulfate, and moisture in grain, as well as a sugar standard for use as a calibrant. Future division activities in the food/nutritional area will be driven by this input.



13. *SRM Activities to Support Health-Care Measurements*

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Objective: To provide certified reference materials that support measurement accuracy and traceability for laboratories performing health-related measurements and *In Vitro* Diagnostic Device (IVDD) manufacturers.

Problem: Inaccuracy in health-related measurements raises overall health care costs, results in misdiagnoses, and leads to inaccurate conclusions in clinical studies. In addition, lack of certified reference materials (CRMs) hampers traceability, and with the new European Community IVDD directive which will require traceability to "standards, of the highest metrological order" there is an urgent need for new health-related SRMs.

The European Community (EC) has scheduled full implementation of a directive on *in vitro* diagnostic devices (IVDD) for December 2003. The directive requires all manufacturers of IVD products sold in Europe to have an "EC Stamp," verifying that they comply with the conditions of the directive. U.S. companies are major exporters of IVDD products to European markets, and thus are directly affected by the directive. One of the major components of this directive is a requirement that products are traceable to "standards of the highest order," *e.g.*, nationally/internationally recognized certified reference materials (CRMs). At present, neither CRMs nor reference methods are available for most of the several hundred analytes that are measured in medical laboratories. These analytes fall into two classes: "A list" analytes that are well-defined chemical species of which there are approximately 80 and "B list" analytes that are less well-defined and number in the hundreds.

One mechanism available on a limited basis for providing the necessary traceability is using clinical reference laboratories to establish traceability to higher order methods. Such laboratories are well established in Europe, but analogous laboratories in the U.S. have generally not been economically viable. With a shortage of clinical reference laboratories in the U.S., it may be difficult for U.S. manufacturers of IVDD products to get the necessary traceability. The European reference laboratory services are very expensive and may not have the reputation to properly address U.S. customer needs. From discussions with representatives of the U.S. IVDD industry, it is clear that they prefer the use of commutable, internationally recognized CRMs as their basis for establishing "traceability to standards of the highest order".

Approach: NIST works closely with the American Association for Clinical Chemistry (AACC), the Centers for Disease Control and Prevention (CDC) and other organizations interested in health-related standards to prioritize our SRM development activities. In general, most health-related SRMs are a matrix of serum or whole blood with certified concentrations of clinically important analytes that have been identified as priorities. In addition to substances normally found in blood, such as cholesterol or calcium, priorities may include toxic substances such as heavy metals, substances believed to improve health status, such as vitamins and other antioxidants, or markers that signal serious conditions, such as the heart attack marker, troponin-I. Once development has begun for a particular SRM, a variety of certification modes may be used, depending upon the measurement needs and NIST capabilities.

CDC identified a need for a whole blood SRM for toxic metals, specifically lead, cadmium, and both inorganic mercury and methyl mercury. They prepared a two-level material from bovine blood with one level representing normal human levels and the other representing elevated levels. The lead concentrations were achieved through feeding of the cows; the elevated levels of the other analytes were achieved through spiking the collected blood material. Certification measurements for cadmium involved using two independent methods at NIST, ID-ICPMS and neutron activation analysis while total mercury was assayed by CV-AAS and ID-ICPMS. Certification of lead was accomplished using the ID-ICPMS definitive method for lead in blood. Value assignment of the methyl mercury involved NIST measurements using GC-atomic emission detection.

Since first issued in 1989, SRM 968 Fat-Soluble Vitamins and Carotenoids in Human Serum has found widespread use in the clinical laboratory community. The measurements for the third renewal of this SRM (SRM 968c Fat-Soluble Vitamins, Carotenoids and Cholesterol in Human Serum) have been completed. Fat-soluble vitamins and carotenoids were measured using two or more NIST methods involving liquid chromatography, along with liquid chromatography methods used by selected laboratories that participate in the NIST Micronutrients Quality Assurance Program. Cholesterol was measured in SRM 968c using the NIST ID/MS definitive method for serum cholesterol.

Troponin-I is a protein that is released into the blood when heart tissue is damaged. Because elevated levels of troponin-I correlate extremely well with a recent heart attack, physicians are now using troponin-I measurements as a diagnostic tool. Unfortunately, different immunoassays for troponin-I produce widely different results. In one large study, the mean from one system was more than 20 times the mean from another. This analyte was identified by the AACC Standards Committee as their highest priority for development of a reference material. NIST is using liquid chromatography/mass spectrometry and MALDI mass spectrometry to characterize purified preparations of troponin-I that are then subjected to testing in laboratories using a variety of routine assays.

Results and Future Plans: Measurements for the lead, cadmium, and total mercury in SRM 966 are complete, and measurements are underway for methyl mercury. Measurements have been completed for SRM 968c which will have certified values for cholesterol, four vitamins, and two carotenoid compounds. Reference values will be provided for nine additional vitamins and carotenoid compounds and information values will be provided for seven additional species. For troponin-I, twelve materials have been evaluated by NIST for their purity and molecular weight distributions. These materials will be sent to a group of laboratories organized by AACC for measurement by the most widely used methods for troponin-I. Those results will be correlated with NIST results and the material judged best will be chosen as a candidate SRM.

We are committed to support the U.S. IVDD industry by maintaining our existing measurement capabilities and serum-based SRMs for calcium, chloride, cholesterol, creatinine, glucose, lithium, magnesium, potassium, nodium, triglycerides, urea, and uric acid. We also plan to intensify both our in-house research program and our interactions with the medical professional and medical laboratory community to develop matrix-based SRMs for the following clinical diagnostic markers over the next 3-5 years:

• Troponin	*heart attack marker*
• Homocysteine	*risk of heart disease*
• Glycated Hemoglobin	*diabetes status*
• Cortisol	*endocrine function*
• Thyroxine	*thyroid function*
• Cadmium	*heavy metal toxicity*
• Folic Acid	*neural tube defects*
• Mercury	*heavy metal toxicity*
• Speciated Iron	*hemochromatosis, anemia*
• Human serum Albumin	*renal failure*
• Prostate Specific Antigen	*prostate cancer*
• Thyroid Stimulating Hormone	*thyroid function*

14. *SRM Activities to Support Environmental Measurements*

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Objective: To provide Standard Reference Materials with value-assigned concentrations of important toxic constituents. These SRMs serve as quality assurance tools for environmental measurement and monitoring efforts worldwide.

Problem: There are a wide variety of environmental monitoring and research programs in the U.S. Inaccurate analytical results lead to inappropriate actions. Therefore accurate analytical measurements are of utmost importance for facilitating sound environmental decision making. Quality assurance programs to enhance the reliability of analytical data often depend upon the availability of a broad spectrum of different control and Certified Reference Materials with matrices similar to the actual environmental samples analyzed.

Approach: For the past twenty years, NIST has provided a large number of natural-matrix SRMs to serve as accuracy benchmarks for environmental measurement and monitoring activities within the U.S. For many matrix and/or analyte combinations, NIST is the *de facto* source for such materials worldwide. Therefore, we are committed to developing new SRMs as needed, as well as recertifying high priority materials as their stocks are depleted and /or providing additional information for important new classes of analytes in both new and existing SRMs.

Results and Future Plans: Certification of a number of new and renewal environmentally-related SRMs has been completed recently. Several SRMs have been certified for both inorganic and organic constituents including:

- SRM 1944 "NY/NJ Waterway Sediment" with certified and reference concentrations of 52 PAHs, 29 PCB congeners, 11 chlorinated pesticides, 17 congeners of 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins and dibenzofurans, and 28 inorganic constituents;
- SRM 1649a "Urban Dust/Organic" with certified and reference values for 44 PAHs, 35 PCB congeners, 9 chlorinated pesticides, 17 congeners of 2,3,7,8-polychlorinated dibenzo-p-dioxins and dibenzofurans, 32 inorganic constituents, mutagenic activity, particle-size characteristics, total organic carbon, total extractable mass, and carbon composition;
- SRM 2977 "Mussel Tissue - Organic Contaminants and Trace Elements," and "SRM 2976 Mussel Tissue - Trace Elements and Methylmercury" both have values for PAHs, PCBs, chlorinated pesticides, trace elements and methylmercury.

SRMs completed this year with certified or reference values for inorganic constituents include:

- SRM 2584 "Trace Elements in Indoor Dust - Nominal 1 % Lead" certified for As, Cd, Cr and Hg;
- SRM 2579a, a set of 5 Lead in Paint films (certified for lead) with a blank film (the films are also individually sold as SRMs 2570-5);
- SRM 1641d "Mercury in Water," SRM 1630a "Trace Mercury in Coal" which has been value assigned for Hg, Cl and S;
- SRMs 2586 and 2587 "Trace Elements in Soil Containing Lead," which have been certified for EPA priority pollutant elements; and
- SRM 2782 "Industrial Sludge" which has also been certified for EPA priority pollutant elements.

SRMs completed recently with certified or reference values for organic constituents include:

- SRM 2978 "Mussel Tissue (Organic Contaminants - Raritan Bay, NJ)" with values assigned for PAHs, PCBs and pesticides;
- SRM 1650a "Diesel Particulate Matter" with certified and reference values for 44 PAHs, as well as particle size characteristics and total extractable mass; and
- two new diesel particulate-related materials, SRM 2975 "Diesel Particulate Matter (Industrial Forklift)" and SRM 1975 "Diesel Particulate Extract" (which is a dichloro-methane extract of the diesel particulate material used in SRM 2975) with certified values for 10-15 PAHs.

The development of a fish tissue material, SRM 1946 "Lake Superior Fish Tissue," is in progress and it will be issued as a frozen tissue homogenate (similar to SRM 1974a and 1945) with certified values for PCBs, pesticides, total mercury, and methylmercury. Other new and renewal environmental SRMs that are currently in progress include SRM 1632c "Trace Elements in Coal" for inorganic constituents; SRM 1566b "Oyster Tissue" which will be certified for elemental content and methylmercury; SRM 2783 "Urban Air Particulate Matter (APM) on Filter for Trace Elements" which contains less than 1 mg of air particulate material per filter and will be value assigned for concentrations of approximately 25 elements; and SRM 270 "Hard Rock Mine Waste which will be value assigned for EPA priority pollutant elements. The supply of some very popular natural matrix SRMs were recently depleted and renewals are currently in progress. These include: SRM 1632b "Trace Elements in Coal"; SRM 1566a "Oyster Tissue"; SRM 1941a "Organics in Marine Sediment"; and SRM 1575 "Pine Needles".

As part of a new program in support of the externalization of the U.S. Environmental Protection Agency's (EPA) Water Supply and Water Pollution Performance Evaluation (PE) studies program, NIST is preparing a number of new calibration solution SRMs for both organic and inorganic contaminants. For the semi-volatile organic contaminants 25 calibration solution SRMs have been prepared including six different Aroclors in methanol and transformer oil; toxaphene and total chlordane in methanol; chlorinated herbicides in methanol; chlorinated pesticides in acetone; haloacetic acids in methyl-*t*-butyl ether; 2,3,7,8 tetrachlorodibenzo-*p*-dioxin in methanol; endothall, glyphosphate, and diquat dibromide in water; chloral hydrate in methanol; carbamates in acetonitrile; and adipate and phthalates in methanol. For the volatile organic contaminants 15 single component calibration solution SRMs have been prepared, all in methanol, at the 1% concentration levels. Six of the solutions have been completed (benzene, toluene, ethylbenzene, *m*-xylene, *o*-xylene, and *p*-xylene (BTEX)). Work on three of the solutions is nearly completed (tetrachloroethylene, carbon tetrachloride, and 1,1,1 trichloroethane), and seven additional solutions have been ampouled and analyses are in progress (1,1-dichloroethene, dichloromethane, 1,2 dichloropropane, 1,2 dichloroethane, 1,2,3 trichloropropane, isopropyl benzene, and *sec*-butylbenzene). Purities of the neat chemicals used to prepare the volatile and semi-volatile solution SRMs have been determined using appropriate techniques (*i.e.*, DSC, GC-FID, GC-MSD, and/or LC), and additional certification measurements will be performed during FY 2000. Six additional solutions not yet identified are also planned for FY 2000. Finally one of five multicomponent mixtures has been prepare in FY99 at the 2000 μg/mL level in methanol. For the inorganic solutions required for the EPA PE program, we will use the existing Single Element Spectrochemical Solution SRM series, and we have initiated the establishment of an NTRM program (discussed elsewhere in this report) to supply these materials.

15. *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*

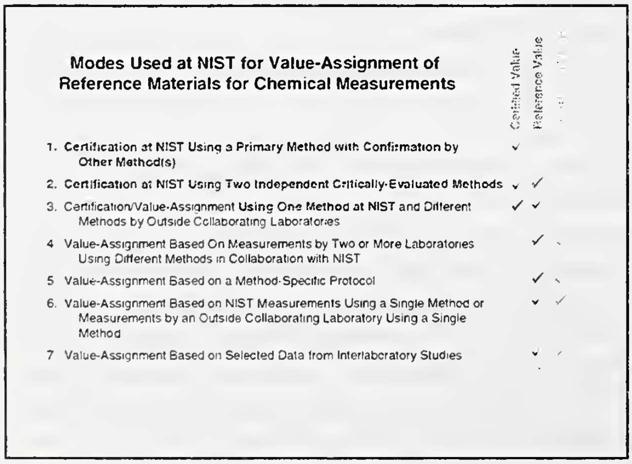
W.E. May, R.M. Parris, C.M. Beck II, J.D. Fassett, R.R. Greenberg, F.R. Guenther, G.W. Kramer, and S.A. Wise; and T. E. Gills, J.C. Colbert, R. Getting, and B. MacDonald (Div. 232)

Objective: To develop a document which provides definitions of terms and descriptions of current practices used at NIST for value-assigning Standard Reference Materials (SRMs) for chemical composition and related properties.

Problem: NIST SRMs are used worldwide for providing quality assurance for chemical measurements. Chemical measurements are becoming increasingly important in international trade decisions and addressing healthcare, environmental and safety-related issues. There has been a recent proliferation of commercial and government-based reference material producers and the current ISO definitions for Reference Materials (RMs) and certified Reference Materials (CRMs). For example, a Reference Material is defined as *a material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials [ISO VIM: 1993, 6.13 [7]]*. A Certified Reference Material is defined as *a Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property* *values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence [ISO VIM: 1993, 6.14].* These definitions say nothing about the quality of the measurement science infrastructure that supports the value-assignment process for RMs and CRMs. This causes confusion for chemical measurement analysts worldwide as they attempt to establish increasingly required traceability for their chemical measurements.

Approach: The quality of assigned values for any CRM or RM is based on the existence and application of sound metrological principles and practices to the value-assignment process. It is with this basic premise that we have developed **NIST Special Publication 260-136**, "Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements". This document provides a complete description of the seven modes used at NIST to acquire analytical data for the value assignment of our SRMs and RMs for chemical measurements and links these modes to three data quality descriptors: NIST Certified Values, NIST Reference Values and NIST Information Values.

Results and Future Plans: A NIST Standard Reference Material® (SRM®) is a CRM issued by NIST that also meets additional NIST-specified certification criteria. NIST SRMs are issued with Certificates of Analysis or Certificates that report the results of their characterizations and provide information regarding the appropriate use(s) of the material. A *NIST Certified Value* is a value reported on an SRM Certificate/Certificate of Analysis for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been fully investigated or accounted for by NIST. Values are generally referred to as certified when Modes 1, 2, or 3 have been used for value-assignment and all the criteria for that mode are fulfilled. These three modes all require NIST measurements and oversight of the experimental design for the value-assignment process. The uncertainty associated with a certified value generally specifies a range within which the true value is expected to lie at a level of confidence of approximately 95 % if the sample is homogeneous. If significant sample heterogeneity is included, the uncertainty generally represents a prediction interval within which the true values of 95 % of all samples are expected to lie at a stated level of confidence.



Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements

	Certified Value	Reference Value	[illegible]
1. Certification at NIST Using a Primary Method with Confirmation by Other Method(s)	✓		
2. Certification at NIST Using Two Independent Critically-Evaluated Methods	✓	✓	
3. Certification/Value-Assignment Using One Method at NIST and Different Methods by Outside Collaborating Laboratories	✓	✓	
4 Value-Assignment Based On Measurements by Two or More Laboratories Using Different Methods in Collaboration with NIST		✓	✓
5 Value-Assignment Based on a Method-Specific Protocol		✓	✓
6. Value-Assignment Based on NIST Measurements Using a Single Method or Measurements by an Outside Collaborating Laboratory Using a Single Method		✓	✓
7 Value-Assignment Based on Selected Data from Interlaboratory Studies		✓	✓

A NIST Reference Value (formerly called Noncertified Value) is a best estimate of the true value provided on a NIST Certificate/Certificate of Analysis/Report of Investigation where all known or suspected sources of bias may not have been fully investigated by NIST. Reference values are generally determined using the following modes:

- Mode 2 or 3 is used when there is lack of sufficient agreement among the multiple methods.

- Modes 4, 5, or 6 are used when the intended use of the value by the measurement community does not require that it be a certified value.

- Mode 7 can be used in special cases, *e.g.*, when results are obtained from another national metrology laboratory with whom NIST has historical comparability data for the method(s) used for the specific matrix/analyte combination.

The uncertainty associated with a NIST Reference Value may not include all sources of uncertainty and may represent only a measure of the precision of the measurement method(s).

A NIST Information Value is considered to be a value that will be of interest and use to the SRM/RM user, but insufficient information is available to assess the uncertainty associated with the value. Typically, the information value has no reported uncertainty listed on the certificate and has been derived from one of the following value-assignment modes:

- Results from modes 4, 5, 6, or 7 in which the intended use of the value by the measurement community does not require that it be a certified or reference value, *e.g.*, information about the

composition of the matrix such as the value of "total organic carbon" of a sediment material may be useful to the user in selecting an appropriate analytical method.
- The results from modes 4, 5, 6, or 7 lack sufficient information to assess the uncertainty.
- Results are provided from outside NIST as supplemental information on the SRM matrix and are not measurements typically made at NIST but may be of interest to the user.

NIST has met the chemical reference materials needs of U.S. industry and commerce for nearly 100 years. Coupled with the fast pace of technological change and greater measurement needs, the demand for additional quantities and additional specific varieties of reference materials has mushroomed. NIST, by itself, does not have the resources to provide SRMs (exact sample types, unique compound combinations, concentrations, etc.) to meet all these needs. Without a significant shift in paradigm, we will not be able to address future needs for reference materials, neither nationally nor internationally. The establishment of these seven modes for value assignment of NIST SRMs and RMs and communication of their linkage to the three quality descriptors (NIST Certified Values, NIST Reference Values and NIST Information Values) is a critical component of our strategy to produce an increasing number of SRMs and RMs through collaborative efforts with selected expert laboratories and other National Metrology Institutes/Standards Laboratories.

Publications:
"Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements," NIST Special Publication 260-136 (1999).

16. *Formal Programs for Providing NIST-Traceable Reference Materials from Commercial Sources*

W.E. May

Objective: To establish formal programs to facilitate the commercial production and distribution of reference materials with a well-defined (and NIST recognized) traceability linkage to NIST.

Problem: Increased requirements for quality systems documentation for trade and effective decision-making regarding the health and safety of the U.S. population have increased the need for demonstrating "traceability-to-NIST" and establishing a more formal means for documenting measurement comparability with standards laboratories of other nations and/or regions. Standard Reference Materials (SRMs) are certified reference materials issued under NIST trademark that are well-characterized using state-of-the-art measurement methods and/or technologies for chemical composition and/or physical properties. Traditionally, SRMs have been the primary tools that NIST provided to the user community for achieving chemical measurement quality assurance and traceability to national standards. Currently, NIST provides nearly 1400 different types of SRMs and in FY98 sold nearly 37,000 SRM units to approximately 5,000 unique customers; approximately 21,000 units of these represent about 850 different types that are certified for chemical composition. NIST does not have the resources necessary to--by itself--provide the increasing quantities and specific varieties of certified reference materials (exact sample types, unique compound combinations, concentrations, etc.) needed to meet increasing needs.

Approach: The NIST Traceable Reference Materials (NTRM) program has been created as part of the solution to this problem. An NTRM is a commercially produced reference material with a well-defined traceability linkage to existing NIST standards for chemical measurements. This traceability linkage is established via criteria and protocols defined by NIST and tailored to meet the needs of the metrological community to be served. Initially the NTRM concept was implemented in the gas standards area to allow NIST to respond to increasing demands for high quality reference materials needed to implement the "Emissions Trading" provisions of the Clean Air Act of 1990 (while facing the reality of constant human and financial resources at NIST). The program has been highly successful and since its inception, ten specialty gas companies have worked with us to certify nearly 4200 NTRM cylinders of gas mixtures, which have been used to produce approximately 400,000 NIST-traceable gas standards for end-users.

The NTRM model for the commercial production of reference materials is being extended to other mature and high volume areas so that more of our resources can be diverted to address new and/or more difficult high priority measurement problems. For the benefit and protection of both the users and providers of these materials, NIST is in the process of trade marking the term NIST Traceable Reference Material (NTRM) in order to restrict its use to only those materials that meet NIST-defined criteria and specifications. Brief descriptions of each of the four areas where NTRM programs are in place or are being developed follow.

Gas NTRM Program *(F. Guenther and W. Dorko)*

The Gas NTRM program was defined to achieve maximum customer confidence in NTRM gas mixture products from Specialty Gas Companies (SGCs). At the time the program was defined it was thought that direct NIST involvement in the certification of the NTRM batches was desirable and necessary. Through this approach we have gained acceptance of these standards by the EPA, the automobile industry, and the stationary source measurement community. We now feel that the program can evolve to lessen the direct involvement of NIST in every NTRM batch. Over the next year we will be defining an alternative certification approach, which will allow SGCs who have demonstrated success through the production of NTRMs over a three-year period. This new approach will certify a particular SGC facility such that any gas cylinder analyzed on a NIST certified analytical system has the potential of being named an NTRM. Adequate controls will be imposed by NIST to assure quality control and traceability to NIST, however the SGC would be able to produce as many NTRMs as they require. It is hoped that this approach will enhance the availability of gas NTRMs such that they will replace gas SRMs as the primary traceability vehicle to U.S. Customers. Another eventual outcome of this new approach would be the worldwide acceptance of gas NTRMs.

Optical Filters NTRM Program *(G.W. Kramer and J.C. Travis)*

NIST has produced Standard Reference Materials for calibrating the wavelength scale and verifying the absorbance accuracy of UV/visible chemical spectrophotometers for several decades. The NIST production capacity is rapidly becoming insufficient to meet the demand, and a recently developed program to leverage NIST measurement capability through the private sector is being adapted to these standards. NIST-Traceable Reference Materials (NTRMs) are produced and marketed commercially, but with the active participation of NIST in the testing and value assignment of the standards. The first NTRMs for chemical spectrophotometry will be on the market within the coming year, and will be modeled on NIST SRM 930e and NIST SRM 1930, neutral density glasses (in a cuvette-simulation format) certified at five wavelengths in the visible spectral region and spanning absorbances between 0.3 and 2.0.

The expanded uncertainties for the certified values will be kept close to those of the corresponding NIST standards by reducing the recertification period from two years to one, which will be compatible with the renewal cycle of many quality control protocols and will provide more frequent cleaning of the filters. The certifying laboratories will be accredited through the NIST-based National Voluntary Laboratory Accreditation Program (NVLAP) and will maintain periodic comparison measurements with the National Reference Spectrophotometer in the Analytical Chemistry Division of NIST. Other spectrophotometric NTRMs for wavelength calibration and UV absorbance verification are expected to follow.

Elemental Solutions NTRM Program *(G. Turk and M. Salit)*

A workshop was held at NIST on June 3, 1999 for major commercial producers of elemental solution standards. The components of a proposed NTRM program were presented to attendees. The key elements of this program include:

-*The introduction of a new series of SRMs.* Elemental Primary Standards, or EPS SRMs.

-*A calibration transfer method that will compare the NTRM against the EPS SRMs.* These measurements would be performed or contracted by the NTRM provider

-*Proficiency testing.* The ability of the NTRM provider to distinguish between slightly different

amounts of analyte in different EPS containers has been proposed, and could be combined with the calibration function by keeping the EPS values unknown to the NTRM providers.
-Software to perform the value assignment of the NTRM with the appropriate uncertainty based on the data of the calibration transfer method. The software (which might be Web-based) will "unlock" the assigned value only if the proficiency test is passed.
-Periodic assessment of NTRM lots by NIST.

We have begun to implement important aspects of this proposal within the framework of the existing SRM Single Element Standard Solutions certification program. We have designated a few NIST Primary (NP) materials, and are preparing and using weighed aliquots of NP solutions (prototype EPS) for value assignment of the Spectrometric Solutions using specially designed high precision ICPOES methods.

Metal Alloy NTRM Development *(J. Fassett and R. Greenberg)*
The first steps in producing metal alloy NTRMs were outlined at a meeting held July 22 and 23, 1999, with the company Analytical Reference Materials International, a secondary metal alloy standards producer which has agreed to work with NIST to pioneer the NTRM concept for metals. A protocol document was prepared and discussed, and the trial process begun, with the intention to "fine tune" the protocol as the process proceeds forward. The company has submitted a proposal to produce 3 NTRM candidate materials:

- AISI Low Alloy Steel
- Chrome-Moly Steel F-11
- AISI 1030 Carbon Steel.

The benchmarks to be used to provide NIST traceability will be from the existing NIST 1700 Series of Low Alloy Steels. Analytical methods to underpin the accuracy of metals SRM and NTRM certification have been developed within the Division in support of the metals NTRM and SRM programs. A quaternary alloy (designated MP35N) has been extensively analyzed within the Division in this development/demonstration exercise. The following techniques have reported results at NIST:

- *Wavelength Dispersive XRF:* Complete elemental analysis, including 19 elements;
- *Glow Discharge OES:* Comparative measurements to the 16 elements determined by industry, including important non-metals (C, P, S, Si, and B);
- *Cold Neutron Prompt Gamma Activation Analysis:* B and C;
- *Instrumental Neutron Activation Analysis:* Majors Constituents (Co, Cr, Mo, Ni) and Mn;
- *ID-TIMS:* S;
- *High precision ICP-OES:* Major Constituents (Co, Cr, Mo, Ni); and
- *Radiochemical Neutron Activation Analysis*: (P).

We need to stress that these capabilities are for the most part new to the Division. Both XRF and GD-OES measurements were made on instruments acquired in the last year with the hope that they could support the metals program, in addition to other programs in the Division. The high precision ICP-OES work was a demonstration experiment that supports our contention that this instrumental technique has primary measurement character—high precision and controlled potential sources of systematic error—and is a candidate replacement for traditional, labor-intensive classical methods. The application of ID-TIMS (for S) and the nuclear methods represent unique capabilities at NIST and are not routinely used by industry. These techniques provide an accuracy benchmark that is also unique, underpinning method-dependent (and standards-dependent) techniques used by industry.

17. *Accreditation of the First Class of Commercial Proficiency Testing Study Providers for EPA/States Water Programs*

R.M. Parris, W.E. May,R.C. Christensen,
E.A. Mackey, J.R. Moody, B.J.Porter, S.D. Rasberry,
T.W. Vetter and C.D.Faison (NIST National
Voluntary Laboratory Accreditation Program)

Objective: To establish a system under which private sector companies and interested states are accredited by NIST to provide proficiency testing (PT) that meets the needs of EPA and states to those laboratories testing drinking water and wastewater for regulated chemical, microbial, and radiochemical parameters.

Problem: Since the 1970's, EPA has conducted semiannual proficiency testing to assess the competence of over 6,000 public and private sector laboratories to conduct analyses required by the Clean Water and the Safe Drinking Water Acts. In 1998, the cost-free provision of these services was phased out to be replaced by a multiprovider system in which interested states and private companies provide these PT services on a fee-basis. Mechanisms and tools to provide appropriate government oversight of these programs were needed.

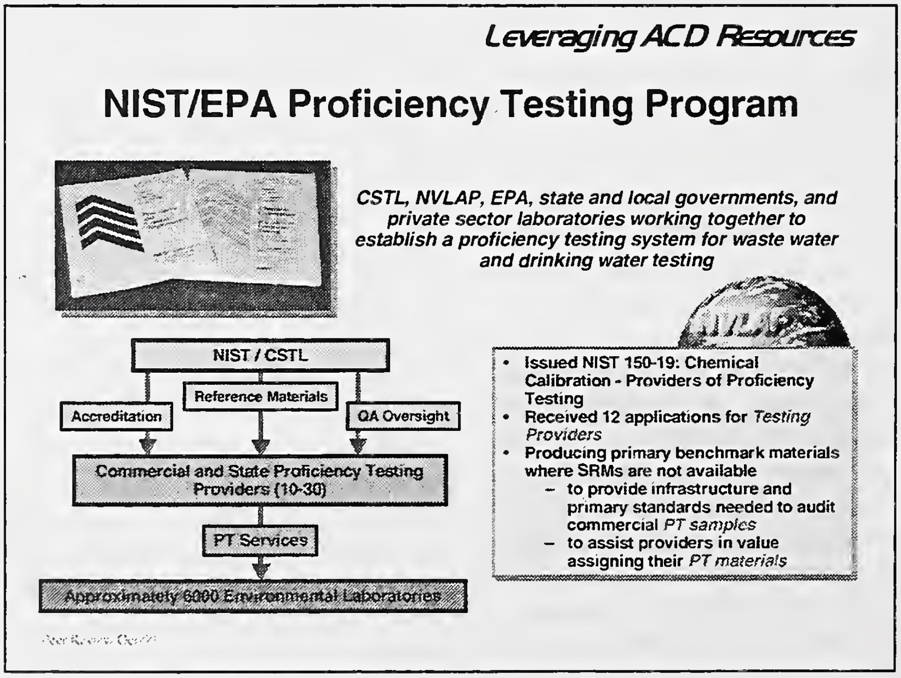
Approach: In a government-private sector partnership, NIST worked with the EPA, States, and other public and commercial entities to establish appropriate oversight of this new effort to externalize and improve the nation's environmental laboratory PT programs. Under this arrangement:

EPA:
- provided NIST with support to develop a program for private sector/state provision of PT studies, and
- works with States and NIST to assure that the program developed for (1) preparing, value-assigning and distributing PT samples and (2) evaluating the quality of the Environmental Testing Laboratory data is sufficient to support national/state water program needs.

NIST:
- developed and manages program for third-party accreditation of private sector water PT study providers,
- establishes and maintains SRMs to support the program, and
- conducts blind sample audits of the commercially supplied PT samples on an ongoing basis as part of our QA responsibility for the program.

PT Study Providers:
- develop, manufacture, value-assign, and distribute PT samples,
- score results of Environmental Testing Laboratory analyses,
- report results to participants, EPA, NIST, States, and appropriate accrediting authorities, and
- maintain accreditation through NIST National Voluntary Laboratory Accreditation Program (NVLAP).

Results and Future Plans: Following extensive discussions among the various stakeholders as to the roles/requirements of the program, a NIST handbook describing the technical requirements of this accreditation program was drafted, presented for comments, and published: NIST Handbook 150-19, Chemical Calibration: Providers of Proficiency Testing. NIST NVLAP accredits laboratories for their competence to characterize samples and to conduct proficiency test programs to support USEPA requirements for environmental laboratories; technical oversight of the program and the evaluation process is provided by the NIST/ACD. In November 1998, NIST NVLAP began accepting applications for accreditation in this new field, Providers of Proficiency Testing, for the provision of chemistry and microbiology PT studies as described in EPA's "National Standards for Water Proficiency Testing Studies: Criteria Document" (US EPA,



Leveraging ACD Resources

NIST/EPA Proficiency Testing Program

CSTL, NVLAP, EPA, state and local governments, and private sector laboratories working together to establish a proficiency testing system for waste water and drinking water testing

NIST / CSTL

Accreditation

Reference Materials

QA Oversight

Commercial and State Proficiency Testing Providers (10-30)

PT Services

Approximately 6000 Environmental Laboratories

- Issued NIST 150-19: Chemical Calibration - Providers of Proficiency Testing
- Received 12 applications for *Testing Providers*
- Producing primary benchmark materials where SRMs are not available
  - to provide infrastructure and primary standards needed to audit commercial *PT samples*
  - to assist providers in value assigning their *PT materials*

December, 1998 Version). NVLAP program designations for providers of radiochemistry PT studies will be added after U.S. EPA has delineated the EPA requirements for these studies in its criteria document.

In October 1999, the first class of nine accredited providers in the Chemical Calibration: Providers of Proficiency Testing program were announced by NIST/NVLAP. Applicant providers still undergoing evaluation will be added to the list on a case-by-case basis as accreditation is granted. A listing of the accredited providers and the specific program codes for which they are accredited are listed in a published directory and on an on-line directory at the NVLAP website, http://ts.nist.gov/nvlap. The current June 1999 version of NIST Handbook 150-19 can also be obtained at this site.

NIST is producing primary benchmark materials such as SRMs for those parameters not covered by existing SRMs to provide the infrastructure needed by NIST to audit the commercially supplied PT samples and to assist providers in value-assigning their PT materials.

18. *Strategic International Collaborations and Comparison Activities*

***W.E. May**, J.D. Fassett, G.W. Kramer,*
M.M. Schantz, M.J. Welch, F.R. Guenther,
K.W. Pratt, and R.M. Parris

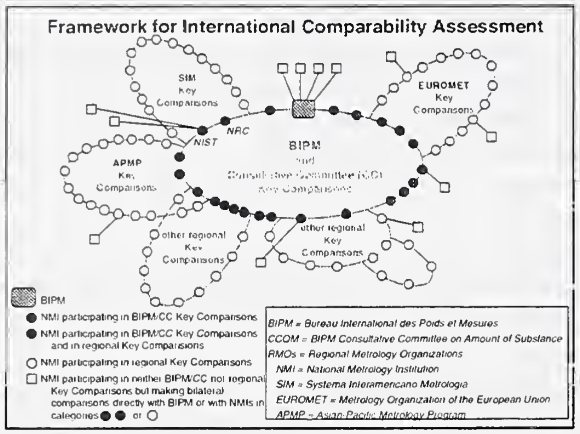
Objective: To assess and document international comparability for chemical measurements among the world's national chemical metrology laboratories and to link such comparability to the U.S. and North American systems of chemical measurement traceability for improved accuracy in chemical measurements.

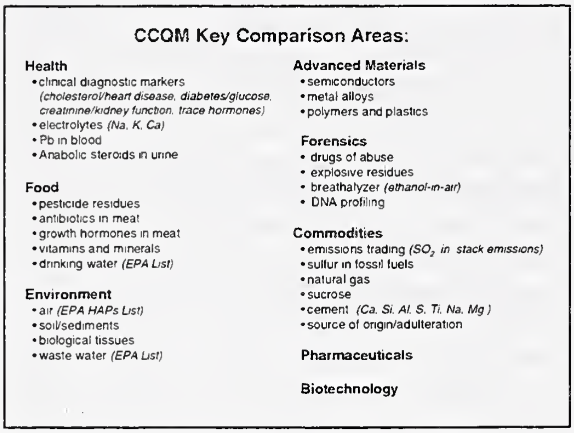
Problem: The need for demonstrating comparability and traceability of chemical analysis data is becoming recognized worldwide. Chemical measurements play a key role in the diagnosis and treatment of diseases, identification of global trends in the state of the biosphere, and the evaluation of the effects of various contaminants in the environment are only possible on the basis of reliable data. A significant proportion of industrial production and international trade is also dependent on chemical measurements. The uncertainty in the validity and/or lack of recognition of many of these measurements leads to a considerable amount of repeated measurements, particularly by regulatory agencies on imported products. This is expensive and impedes the free flow of international trade.

Approach: In October of 1999, nations and economies signed a "Mutual Recognition Arrangement between National Metrology Institutes for recognition of National Measurement Standards and of Calibrations, and Measurement Certificates. The Analytical Chemistry Division is a key participant in efforts of the International Committee for Weights and Measures (CIPM) and its Consultative Committee on Amount of Substance (CCQM) to address the issues of comparability and traceability for chemical measurements. The CCQM has begun to assess chemical measurement comparability through strategically selected comparisons among national chemical metrology laboratories in the general areas of advanced materials, biotechnology, commodities, the environment, food, forensics, health, pharmaceuticals, and general analytical applications. The Division has participated in all 15 of the comparisons that have been undertaken to date. The Division is also using the assessment exercises conducted through the CCQM for selecting partners for strategic bi- or trilateral collaborations to determine and document the comparability of NIST primary methods and standards with those of other nations and/or metrological regions. The Division holds the Chair for the Chemical Metrology Working Group within the Interamerican System of Metrology (SIM) and past chair for the NORAMET subregion.

Results and Future Plans: The CCQM has formed five working groups: (1) gas analysis, (2) organic analysis, and (3) inorganic analysis, (3) pH and Conductivity, and (4) Key Comparisons. These working groups are responsible for selecting and overseeing the operation of key comparisons that address chemical measurement-related issues important for international trade, environmental, health, and safety-related decision making. The Division is very active and has led various activities within all five working groups.

The Division provides the official leadership for the chemical metrology activities within SIM. Because the capabilities of the 34 countries within SIM span such a broad range, activities have initially been focused on training and capability assessment. Since only 3 of the 34 countries have formal programs in chemical metrology, we conducted the following courses during the past year for current or designated future leaders of chemical metrology programs within SIM:





CCQM Key Comparison Areas:

Health
- clinical diagnostic markers *(cholesterol/heart disease, diabetes/glucose, creatinine/kidney function, trace hormones)*
- electrolytes *(Na, K, Ca)*
- Pb in blood
- Anabolic steroids in urine

Food
- pesticide residues
- antibiotics in meat
- growth hormones in meat
- vitamins and minerals
- drinking water *(EPA List)*

Environment
- air *(EPA HAPs List)*
- soil/sediments
- biological tissues
- waste water *(EPA List)*

Advanced Materials
- semiconductors
- metal alloys
- polymers and plastics

Forensics
- drugs of abuse
- explosive residues
- breathalyzer *(ethanol-in-air)*
- DNA profiling

Commodities
- emissions trading *(SO_2 in stack emissions)*
- sulfur in fossil fuels
- natural gas
- sucrose
- cement *(Ca, Si, Al, S, Ti, Na, Mg)*
- source of origin/adulteration

Pharmaceuticals

Biotechnology

Organic Analytical Metrology; December, 1998;
12 participants
Spectrochemical Metrology;December, 1998;
14 participants
Nuclear Analytical Metrology; April, 1999;
6 participants
Classical Methods; May, 1999; 13 participants
Gas Metrology; June, 1999;10 participants

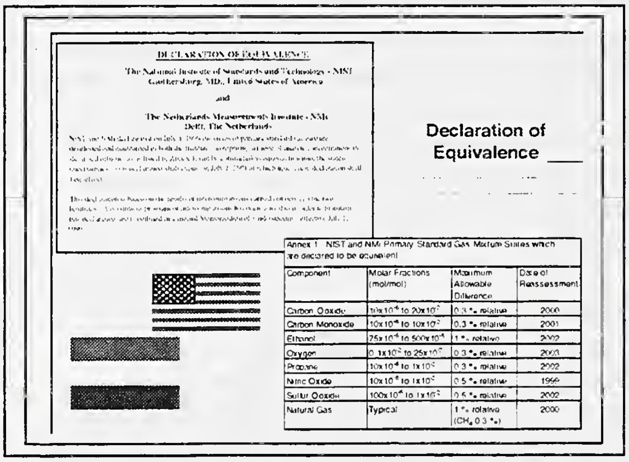
We also identified six comparison exercises to test the proficiency of NMI's or their designated collaborators for addressing chemical measurement problems within the Americas.

• *SIM-QM-P1:*	Chlorinated Pesticides in Organic Solvent.
• *SIM-QM-P2:*	Trace Metals in Drinking Water
• *SIM-QM-P3*:	Automotive Exhaust Emission Gases
• *SIM-QM-P4:*	pH (5-7 range)
• *SIM-QM-P5:*	Vitamins and Minerals in Infant Formula
• *SIM-6:*	Holmium oxide

The NORAMET subregion of SIM consists of NIST, NRC-Canada, and CENAM-Mexico. All three institutes produce Certified Reference Materials (CRMs) and have agreed to extract comparison data while assisting in each other's reference materials certification campaigns. The following comparisons took place during the past year using this paradigm:

- Metals in Drinking Water [CENAM]
- Elements in Sediments and Mussel Tissue [NRC]
- Organics in Sediments and Fish Tissue [NIST]
- Methyl Hg in Fish Tissue [NIST]
- Vitamins and Minerals in Milk Powder [CENAM]

For six years we have been involved in a strategic bilateral program with the National Measurement Institute (NMi) of The Netherlands for determining the equivalence of primary gas standards. Our activities have resulted in a formal "Declaration of Equivalence" that is mutually recognized by the U.S. EPA and European environmental regulatory bodies as documenting the equivalence of eight NIST and NMi primary gas mixtures suites (spanning a wide range of concentrations). In previous years the carbon dioxide, carbon monoxide, ethanol, oxygen, propane, nitric oxide, and sulfur dioxide PSMs have been shown to be equivalent. During the past year we have worked to establish equivalence in natural gas standards and have continued efforts to address a bias of 2% observed between NIST and NMi primary standards for hydrogen sulfide. Additional collaborations for assessing



Declaration of Equivalence

DECLARATION OF EQUIVALENCE

The National Institute of Standards and Technology - NIST
Gaithersburg, MD., United States of America

and

The Netherlands Measurements Institute - NMi
Delft, The Netherlands

Annex 1 NIST and NMi Primary Standard Gas Mixture Suites which are declared to be equivalent

Component	Molar Fractions (mol/mol)	Maximum Allowable Difference	Date of Reassessment
Carbon Dioxide	$10x10^{-6}$ to $20x10^{-2}$	0.3 % relative	2000
Carbon Monoxide	$10x10^{-6}$ to $10x10^{-2}$	0.3 % relative	2001
Ethanol	$25x10^{-6}$ to $500x10^{-6}$	1 % relative	2002
Oxygen	$0.1x10^{-2}$ to $25x10^{-2}$	0.3 % relative	2003
Propane	$10x10^{-6}$ to $1x10^{-2}$	0.3 % relative	2002
Nitric Oxide	$10x10^{-6}$ to $1x10^{-2}$	0.5 % relative	1999
Sulfur Dioxide	$100x10^{-6}$ to $1x10^{-2}$	0.5 % relative	2002
Natural Gas	Typical	1 % relative (CH_4 0.3 %)	2000

the equivalence of primary standards have been established with DFM (Denmark) and OHM (Hungary) for conductivity, with the PTB for pH, with EMPA (Switzerland) for elemental solution standards, with NIMC for volatile organic compound standards, with INPL (Israel) for moisture in oils, and with NPL (UK) for optical absorbance filter standards.