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Chemical Science and Technology Laboratory

Technical Activities
1996

U.S. DEPARTMENT OF COMMERCE
Technology Administration
National Institute of Standards and Technology

Submitted to the Board on Assessment of NIST Programs,
National Research Council

Shown on the cover (clockwise starting at the bottom):

A data screen from the NIST Mass Spectral Database, showing likely matches between the mass spectrum of an unknown compound with several compounds from the database — this database will soon contain over 120,000 fully-evaluated entries. This is one of many important data activities in CSTL which provide critical data resources needed by industry and the scientific community.

An x-ray spectrum of a yttria-stabilized zirconia film, measured by electron microprobe wavelength-dispersive analysis. A series of these x-ray spectra measurements, taken across a cross-sectional area of the film, allow the construction of a compositional map. These microanalytical measurements and methods enable the study of films and coatings important to U.S. industrial quality and competitiveness.

The characterization of Standard Reference Materials (SRMs) is a core activity of CSTL. Shown is an ampoule of SRM 2294 (Reformulated Gasoline) used for the quantitative analysis of oxygenates in fuels. The nation's traceability structure, for measurements in chemistry, chemical engineering, and biotechnology, is based upon the availability of critically-evaluated measurement standards.

The development of critical biochemical measurements and methods advances the scientific basis and societal impact of biotechnology. The study and manipulation of the DNA molecule is one such focus in CSTL. Well-characterized materials have been produced that enhance quality assurance procedures of genetic testing (DNA Fingerprinting). Slab gel electrophoresis methods have been developed to detect single DNA-base mutations in the human p53 (anti-tumor) gene.

A micro-machined, thin-film gas sensor array (microhotplate) has three functional layers: a heater, a thermometer/heat distribution plate, and sensing film electrical contacts. These devices are being developed to provide inexpensive, real-time measurement technology with broad industrial applications, e.g., in process control, chemical identification, and auto emissions measurements. The view across the image is about 500 micrometers.

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

Hratch G. Semerjian, Director
Chemical Science and Technology Laboratory
National Institute of Standards and Technology

U.S. DEPARTMENT OF COMMERCE, William M. Daley, Secretary
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Chemical Science and Technology Laboratory

Hratch G. Semerjian, Director
William F. Koch, Deputy Director
W. Mickey Haynes, Associate Director for Boulder

Program Overview

Since its creation by Congress in 1901, NIST (the National Bureau of Standards at that time), has been the Nation's leading reference laboratory for measurements and standards to support U.S. industry, commerce, scientific institutions, and all branches of Government. NIST's unique mission is to promote U.S. economic growth by working with industry to develop and apply technology, measurements, and standards. The NIST laboratory research program, of which the Chemical Science and Technology Laboratory (CSTL) is a part, is planned and implemented in cooperation with industry and focused on infrastructural technologies such as measurements, standards, evaluated data, and test methods.

Mission:
As the Nation's Reference Laboratory, 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 chemical, biomolecular, and chemical 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 achieve its vision by setting and meeting the following goals:

- 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.
- 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.
- Anticipate and address next generation measurement needs of the nation, by performing cutting-edge research in measurement science.

CSTL is a multi-faceted organization with a broad customer base. We have state-of-the-art technical capabilities and expertise in analytical chemistry, surface chemistry and microanalysis, biotechnology, physical, and chemical properties, process measurements and modeling. The needs of our customers are reflected significantly in our program priorities. Customers purchase our products and services (such as Standard Reference Materials, Standard Reference Data, or calibration services), collaborate with us through consortia and CRADAs, contract for our services, and interact through round robins, conferences, workshops, and informally with our technical staff. Our primary customers are: industry (chemical, electronics, automotive, petroleum refining, instrumentation, biotechnology, environmental technologies, and health care); universities; federal, state, and local government agencies; and standards and industrial trade organizations. Accomplishments in FY 1996 are summarized in the table below.

Organizational Structure:
To achieve the laboratory's technical goals, CSTL maintains an experienced, well-educated professional staff. The permanent staff numbered 274 in FY 1996 with an additional 90 temporary and part-time employees. Also, there were almost an equal number (276) of guest researchers. The technical staff consisted of 119 chemists, 40 physicists, 36 engineers, 16 biologists or computer specialists, and 29 technicians.

Div	Pubs.	Talks	Committees[1]	Seminars	Conferences	CRADAs	Patents	SRMs	SRDs	Cals.[2]
830	1	9	37	11	0	0	0	0	0	0
831	89	131	50	27	4	9	0	1	1	0
836	73	94	103	24	5	16	3	3	0	769
837	89	108	57	8	10	0	0	9	4	5
838	179	92	104	44	3	5	0	3	11	27
839	134	131	85	9	11	18	0	144	2	264
Totals	565	565	436	123	33	48	3	160	18	1065

[1]Committee totals include 45 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

Some important changes were made in the CSTL management structure in FY 1996. At the end of 1995, the Chief of the Chemical Kinetics and Thermodynamics Division, Sharon Lias, retired. The division was combined with the Thermophysics Division, under the leadership of Richard Kayser to become the Physical and Chemical Properties Division. In addition, the Pressure and Vacuum Group was moved from what was the Thermophysics Division to the Process Measurements Division.

Research Program Assessment:
We balance our programs between essential measurements technology and basic research 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. As part of our strategic planning process, we assess and prioritize future needs and re-orient our research program accordingly. In FY 1996 we began or enhanced seven new programs:

- computational chemistry,
- international comparability of chemical measurements,
- molecular scale materials characterization,
- national traceability and international comparability for physical measurements,
- health care measurements,
- bioinformatics, and
- evaluated process design data.

This re-orientation of CSTL's research effort is being done with existing resources.

Traceability and International Comparability:
The characteristics of international trade and industrial development are rapidly changing, becoming more global and more competitive. As regulatory barriers are reduced, technical barriers, associated with mutual acceptance of products, materials, and services, are becoming more important. As a result, there is more emphasis on conformity assessment issues including product quality certification, calibration and testing laboratory accreditation, and quality systems registration. Fundamental to these issues are the technical and metrological comparability needs that affect product testing standards and performance measures inherent in the accreditation process.

CSTL has developed close working collaborations with the major national standards laboratories for specific physical and chemical measurements around the world. As a result, we have established bilateral and multi-lateral cooperative agreements with our counterparts, and are participating in the prioritization, organization, and execution of key measurement intercomparisons. These activities are designed to form **the basis of declarations of equivalency** for specific measurement parameters and standards.

For example, we have completed the effort to provide and disseminate the International Temperature Scale of 1990 (ITS-90) over the range of 0.65-1235 K by completing the **gas thermometry work from 3 K to 24.6 K.** NIST leads the world in both the temperature range and level of uncertainty in its realization of ITS-90 (836).

The Surface and Microanalysis Science Division has recently completed the development of **isotopic standards for carbon and oxygen in CO_2.** These three reference material will be analyzed by thirteen laboratories to establish internationally recognized reference values for natural ^{13}C and ^{18}O abundances.

The Analytical Chemistry Division has renewed and updated the Declaration of Equivalence with the **Netherlands Measurement Institute** for primary gas standard mixtures. This Declaration is supported by an ongoing program of intercomparisons. In FY 1996, three additional gas mixtures were shown to be equivalent, bringing the total number of equivalent mixtures to seven.

We have also established a Memorandum of Cooperation among NIST, the **Danish Institute of Fundamental Measures, and the Hungarian National Office of Measures** to establish intercomparisons which will lead to equivalency statements for electrolytic conductivity measurements (839).

Under the auspices of the **Consultative Committee for the Amount of Substance (CCQM) for the CIPM,** NIST has organized an intercomparison on the determination of lead in water as a follow-up study to the 1994-5 inorganic analysis intercomparison using isotope dilution mass spectrometry (IDMS) as the primary method. NIST provided a detailed protocol for using an inductively-coupled plasma mass spectrometer system with the IDMS method to improve the accuracy and variability of results from the first intercomparison. These results are due in FY 1997.

NIST is also a participant in the CCQM intercomparison for the IDMS determination of pesticide metabolite, pp′-DDE, and gas mixtures. These results are to be reported early in FY 1997 (839).

NIST chairs the International Working Group for Humidity Measurements (IWGHM) under the auspices of the **Consultative Committee on Thermometrie (CCT) of the CIPM.** The IWGHM has planned and is conducting intercomparisons of precision hygrometers in national laboratories in three regions, the European Union, the Asian-Pacific, and the North American region. Regional inter-compar-isons have begun in the European and Asian-pacific regions. A small number of key intercomparisons between regions have been identified. CSTL is participating in these.

International intercomparisons and declarations of equivalence are facilitated by metrology efforts coordinated at the regional level. As a member of NORAMET, the North American Metrology cooperative organization, NIST works closely with the National Research Council (NRC) of Canada and the Centro Nacional de Metrologia (CENAM) of Mexico. NIST organizes the NORAMET activities related to analytical chemical measurements. In FY 1996, NIST and NRC completed an intercomparison on trace metals and PCBs in marine tissue and sediment materials. NIST has also provided extensive training to 14 CENAM scientists in a variety of areas of analytical chemistry (839).

Technical Achievements:
There were many noteworthy technical achievements by CSTL scientists and engineers during FY 1996, examples of which are presented below. More detailed information about these activities can be found in the appropriate Division's selected technical highlights.

In FY 1996, CSTL staff produced a number of outstanding research accomplishments serving a great diversity of constituents. The Biotechnology Division has completed measurements of the **kinetics and specificity of DNA repair by certain enzymes.**

The use of the NIST/NASA/CARB **Biological Macromolecular Crystallization Database (BMCD)** has been greatly facilitated by its deployment on the World Wide Web. Users can now browse, search, display, and download data for developing crystallization protocols for newly isolated biological macromolecules (831).

The **high-resolution separation of different physical forms of DNA** has been demonstrated using electrochromatography. Control of the separation is achieved by varying the electric field strength, flow rate and stationary phase porosity. This approach holds greater promise for scale-up than the laboratory-scale electrophoresis approach. Two patent filings were made in FY 1996 as a result of this research (831).

The first **images of self-assembled monolayer (SAM) growth** have been produced for alkane thiol monolayers on gold. By varying the degree of gas dosing, direct STM images show a three-stage progression from a two-dimensional lattice-gas phase to the final high-density solid phase SAM. This approach provides a key element in understanding the processes by which useful sensors, lubricants and optical films based on organic monolayers can be developed (836).

The **combination of gas chromatography and accelerator mass spectrometry** was used by the Surface and Microanalysis Science Division, in collaboration with Woods Hole Oceanographic Institute, to separate and provide radiocarbon dating on individual trace organic compounds. This achievement in extremely small environmental samples is the first step towards the capability to distinguish anthropogenic sources of organic compounds from those occurring naturally in the environment.

Precise measurements of the $^{34}S/^{32}S$ isotopic ratio have been made by the Analytical Chemistry Division for some 60 samples of atmospheric particles collected from the Mount Zirkel Wilderness Area. Our unique capability to measure such ratios to a relative expanded uncertainty of less than 0.2% was used as part of a collaborative study with the Desert Research Institute of the University of Nevada. The study is designed to differentiate among several possible natural and anthropogenic sources of smog that are degrading the visibility in the area.

The Surface and Microanalysis Science Division demonstrated the use of **electron microprobe wavelength dispersive compositional mapping** methods for determination of compositional variations in yttria-stabilized zirconia (YSZ) coatings. This provides the coatings industry with a method to determine the effectiveness of bonding layers used with YSZ coatings on high temperature surfaces, e.g., aircraft turbine blades.

Development of **optical diagnostic methods to probe buried interfaces in semiconductor materials** has been demonstrated in the Surface and Microanalysis Science Division. Second harmonic and sum frequency generation techniques have been used to study Schottky barriers formed between Si (111) and (100) oriented substrates and $CoSi_2$. Twinned interfaces were observed and localized defect states were found in Au/GaAs barriers. Schottky barrier structures are fundamental to fabrication of high speed bipolar semiconductor devices.

The morphology and microstructure of 50 nm TiO_2 particles has been determined with **electron holography** by the Surface and Microanalysis Science Division. Particles formed in isopropoxide flames in the Process Measurements Division have central features that are faceted voids.

The Physical and Chemical Properties Division has developed an absolute **Greenspan acoustic viscometer**, which promises to provide a robust means for measuring the viscosities of the reactive and toxic gases used in semiconductor manufacturing and the noble gases used in thermo-acoustic refrigerators.

The Analytical Chemistry Division has evaluated the limits of **precision and accuracy of the wavelength scale** in UV/visible fourier transform spectroscopy (FTS), in tandem with a novel optical coupling scheme, has resulted in a system capable of making broadband wavelength measurements with unprecedented accuracy. This system, employing a 2-color clock-based frequency standard as a calibrant, is being used to re-measure the wavelengths of ^{198}Hg emission lines with relative expanded uncertainties of about 1×10^{-8} of the wavelength. Improvements in total expanded uncertainty of the wavelength scale to match the sub-part in 10^9 precision are being investigated.

Measurement Services:
CSTL provided 76% of the Standard Reference Materials (SRMs) and 13% of the calibration services provided by NIST. CSTL also provided 60% of the databases provided by the Standard Reference Data (SRD) Program in FY 1996. 3864 copies of the mass spectral database were sold, the majority going to manufacturers of mass spectrometers as part of the spectrometer system. In FY 1996 the Process Measurements Division completed the automation of calibrations for liquid-

in-glass thermometers, commonly used platinum resistance thermometers, thermistor thermometers, and thermocouples. For pressure and vacuum measurements, we have extended the range of our high-pressure calibration capabilities from 16 Mpa to 280 MPa. A new gravimetric hygrometer was developed, capable of measurements with ±0.1% relative standard uncertainty in the range of -42 °C to 40 °C.

NIST chemical properties data are now available on the Internet with the first release of the Chemistry WebBook. Thermodynamics data for over 5000 compounds and ion energetics data for over 7000 compounds are now widely available through automated searches by compound name and by full or partial formula. Version 2.0 of the NIST/ASME database on Thermophysical Properties of Water is now complete. This version now fully implements the new formulation for thermodynamic properties adopted by the International Association of Water and Steam in 1995 (838).

The Analytical Chemistry Division has completed ultra-high accuracy determination of sulfur in a key alloy used by the aircraft industry to ensure the reliability of jet engine blades. These new measurements led to the re-certification of SRM 131e, High Silicon Low Alloy Steel with a reduction in the sulfur concentration uncertainty of a factor of 20.

Just as new regulations on leaded paint disclosure were put into effect by the EPA, NIST completed a series of lead in paint, soil, and dust SRMs to ensure that environmental and health decisions have a reliable accuracy base for lead determinations (839).

CRADAs, Consortia, and ATP-Collaborations:
The Process Measurements Division has commissioned its new calibration and testing facility for gas flow meters for vehicle exhausts as part of our CRADA with the American Industry/Government Emissions Research (AIGER) consortium. This **exhaust meter calibration facility** is the first of its kind, generating simulated automobile exhaust at known flow rates with controlled temperatures and moisture content. The first tests of industrial meters are underway. To assist AIGER in addressing compliance issues associated with the Clean Air Act of 1990, the Analytical Chemistry Division is developing an **infrared-based sensing device** and working with the NIST Physics Laboratory to evaluate a new microwave device **for detection of oxygenated hydrocarbons** in automobile exhaust. The use of oxygenated and reformulated fuels has been required by a number of metropolitan areas in order to comply with Federal air quality standards. Gasoline standards that are currently under development include a suite of **synthetic gasoline materials** being **certified for benzene, total aromatics, total oxygen, total olefin, and sulfur content**. These SRMs will provide NIST traceability for the new reformulated gasolines. Another related standards activity involves the development of catalytic conversion technology for the on-demand **generation of standard formaldehyde-in-air mixtures** from methanol-in-air standards. A prototype device is currently being field tested in an automobile industry facility.

The Biotechnology Division interacts with a number of industrial CRADA partners. A CRADA agreement established with Life Technologies, Inc. has applied long polymerase chain reaction (PCR) technologies to the analysis of variable number of tandem repeats in human DNA identification. The results of this work demonstrate a means to link the commonly used forensic database CODIS, based on restriction fragment polymorphism analysis (RFLP), with PCR which is replacing RFLP as the most commonly used technique to analyze DNA. The ability to convert the RFLP assay system to a PCR-based system will result in substantial savings to the human identity community of both time and labor.

CRADAs in the Physical and Chemical Properties Division address the development of both the **world's smallest and largest pulse tube refrigerators**. In cooperation with Cryenco, Inc. of Denver, CSTL has tested a redesigned module of the largest cryocooler for liquefying natural gas, achieving a no-load temperature of 55 K. Based on this collaboration, a 500 L/d liquefier is now under construction at Cryenco. A collaboration with Lockheed Martin of Denver has resulted in the design and construction of a miniature two-stage pulse tube refrigerator for use on a future space shuttle mission. The laboratory prototype can achieve a no-load temperature of 76 K.

Several of our activities directly support the biotechnology-focused area in the Advanced Technology Program. Reference material development for DNA diagnostics has led to more efficient methods for detecting mutations associated with genetic diseases. Capillary electrophoresis methods of mutation detection in the p53 gene have been developed. The "wild-type" of this gene has been associated with control of cell growth. Loss of its activity is often found in human cancer development. The cloning of a DNA polymerase was also accomplished, in an effort to decipher the nucleotide sequence of various genomes and individual. This problem is especially relevant because the bacteria has a high guanine-to-cytosine ratio (70:30) which should allow better sequencing of high guanine-to-cytosine DNA samples.

Patents:
In FY 1996 three patents were awarded to CSTL researchers and twelve were filed. Two biotechnology-related patents were awarded. **Photochromic Compositions and Materials Containing Bacteriorhodopsin (bR)** deals with materials that increase the conversion efficiency of optical energy into metabolic energy (831). **Process for UV-Photopatterning of Thiolate Monolayers Self-Assembled on Gold, Silver and Other Substrates** describes a lithographic process to transfer patterns into alkanethiol monolayer films. This has potential for application to biosensor arrays utilizing molecular recognition events (836). **Thin-Film High Temperature Silicide Thermocouples** describes thermocouples of $TiSi_2$ and $MoSi_2$ that are reliable in high temperature applications, have fast response times, and may be fabricated to provide greater spatial resolution than current wire-based technologies (836).

Conferences and Workshops:
The NIST/NCI Quality Assurance (QA) Workshop was held in Washington, DC for laboratories that measure vitamins in human serum. This annual workshop is part of a QA program supported by the National Cancer Institute (NCI) to study the possible cancer preventive benefits of vitamins in human populations at high risk of developing certain forms of cancer. The workshop, attended by approximately 60 researchers and technicians in FY 1996, serves as the principal means for participants to discuss analytical methods, results of the intercomparison exercises for the past year, and other related thrust areas such as methods analysis for bioflavonoids and selenomethionine in human serum. For laboratories involved in the chemoprevention proficiency studies, NIST staff also provide the participants with technical feedback concerning their performance and suggestions for method development and refinement. As a result of the workshop, the participating laboratories' measurement capability for retinol, alpha-tocopherol, and b-carotene has substantially improved. Increased accuracy has been and can be achieved with the use of proficiency test samples, SRMs, and control materials, and with consultation in methods development and analytical-measurement training sessions.

A workshop on the Thermophysical Properties of Ammonia/Water Mixtures was held in Boulder, CO to provide a government-industry forum on the development and use of this important type of working fluid. Major objectives of the meeting were to determine which thermophysical properties are required to advance the technologies based on this mixture and to establish a mechanism for obtaining the required data and models. CSTL staff and industry experts exchanged information on current activities and future needs. There was a strong consensus that thermophysical properties over a broad range of temperature, pressure and composition are vitally important for the design stages of power plant development based on ammonia/water working fluids. It was further concluded that filling in missing data was of a higher priority than improving the accuracy of existing data. As the technology matures, better models will be requires to improve and optimize plant designs.

Awards and Recognition:
Many CSTL scientists received awards and recognition. J.M.H. Levelt-Sengers was elected a **Fellow of the National Academy of Sciences** in recognition of her work on the properties of fluids and fluid mixtures over wide ranges, including the critical point. Donald Archer received the **1996 Stig Sunner Award of the Calorimetry Conference** for his work correlating the dielectric constant of water, on the thermodynamic properties of salt and surfactant solutions, and on enthalpy-increment measurements over wide ranges. Hratch Semerjian was elected a **Fellow of the American Society of Mechanical Engineers**. William Koch was elected

President (Elect) of the **National Committee for Clinical Laboratory Standards**. Charles Tilford was elected a **Fellow of the American Vacuum Society**. Donald Becker was elected a **Fellow of the American Nuclear Society**. Michael Kurylo received the **NASA Exceptional Service Award**. Mark McLinden was recognized as the **Department of Commerce/NIST Engineer of the Year**. Steve Stranick received **the Nobel Laureate Signature Award of the American Chemical Society** for "... inventing the tunable microwave frequency ac scanning tunneling microscope ... the first of its kind to obtain chemical information on the surfaces of insulating, as well as conducting, solids". Eric Steel, Shirley Turner, Jennifer Verkouteren, and Eric Windsor received **NIST's 1996 Allen V. Astin Award** in recognition of their outstanding work in "providing the technical basis for measurements of bulk and airborne asbestos". They also developed and certified 6 SRMs critical to the accreditation of more than 700 commercial testing laboratories who make asbestos measurements for asbestos abatement projects that protect the public, particularly schoolchildren. Two 1996 **DoC Silver Medals** were awarded to CSTL staff members. Richard Kayser was recognized for "his outstanding technical and managerial leadership ... in the development of the world's pre-eminent program on the thermophysical and chemical properties of fluids and fluid mixtures...". Also, Gary Gilliland was recognized for "leadership of the unique joint program between NIST and the University of Maryland Biotechnology Institute at CARB" and for "his world renowned research in protein crystallography". Two CSTL staff received **DoC Bronze Medals**; Malcolm Chase for " leadership on new dissemination methods for NIST Standard Reference Data in Computerized and published formats", and Gregory Gillen for "his fundamental research in measurements and standards and leadership role in developing novel applications of secondary ion mass spectrometry". Rhoda Levin received **the NIST Equal Employment Opportunity Award** her efforts in "crafting, propelling, and promoting the NIST Mentoring Program". **1996 Measurement Services Awards** went to Stephen Doty in recognition of his efforts in establishing a new calibration service for high-pressure-gas piston gauges, Allan Harvey for development of a new database for the thermophysical properties of water and steam based on the new international standard, John Houser for his efforts in establishing a new volumetric test measure calibration facility, and Stephen Wise for establishing the joint public-private sector research and measurement programs leading to rapid SRM development. Gregory Poirier received the **CSTL Technical Achievement Award** for his research on the self-assembly mechanisms of alkanethiols on gold. The NIST Chapter of Sigma Xi recognized Edgar Etz for his **Outstanding Service to the Society** and Frank DiMeo for the **Outstanding Poster Presentation** at its Annual Postdoctoral Symposium.

Technical Highlights

In the following pages, recent activities and technical directions of the CSTL Divisions are highlighted.

I. Biotechnology Division (831)

Walter J. Stevens, Chief

A. Division Overview

The Biotechnology Division is the focus of the NIST effort 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. The Division fosters collaboration among NIST scientists conducting biotechnology research and raises the visibility of the NIST Biotechnology program, which leads to enhanced collaborations with industries, universities, and other government agencies.

To plan and guide Division research programs effectively, Division scientists are active in many forums that provide feedback on long-term and short-term needs for biotechnology commercialization. These activities supplement the high level of Division participation in scientific meetings and topical workshops. For example, during FY 1996 the Division continued active participation in the IUPAC Commission on Biophysical Chemistry, the ASTM Committee E-48 on Biotechnology, and the Biotechnology Industry Organization (BIO). Division members were also active participants in a number of important workshops held at NIST, i.e., “The Sixth International Meeting on Chemical Sensors.” 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 participated in ATP sponsored workshops and public meetings presenting the results of Division research programs. Additionally, it should also be noted that Division members have been involved in generic 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 coordination of Federal biotechnology research through membership in the Biotechnology Research Subcommittee (BRS) of the National Science and Technology Council (NSTC).

The staff of the Biotechnology Division consists of 44 NIST employees and a comparable number of contract researchers, guest scientists, and postdoctoral fellows. The Division is organized in four groups: 1) DNA Technologies, 2) Bioprocess Engineering, 3) Structural Biology, and 4) Biomolecular Materials. In FY 1996, the Biomolecular Materials group was formed from the Biosensor Technology group. During the course of the year, the Division management has remained stable. Near the end of the year, however, Dr. Walter Stevens, the Division Chief, announced that he would be leaving the Division. Dr. Gary Gilliland, the Structural Biology Group Leader and Associate Director of CARB is assuming the role of Division Chief for FY 1997. Dr. Edward Eisenstein, a University of Maryland Biotechnolgy Institute faculty member, will become the Structural Biology Group Leader and Associate Director of CARB for FY 1997.

FY 1996 was a very productive year for scientists of the Biotechnology Division. A brief overview of each Group in the Division, and highlights of several research programs are given below:

The **DNA Technologies Group** carries out research in DNA and molecular biology to enhance measurement technologies and to provide standard reference materials (SRM) for application in areas related to the detection and characterization of DNA. Interlaboratory tests that are designed to evaluate methods for DNA-based human identification have been conducted by the Group each year for the past several years. Standard Reference Materials have been developed to allow accurate characterization of DNA profiles for forensic and paternity testing. Expansion of standards development for **mitochondrial DNA** testing has been the focus of work in 1996. This has resulted in the development of a prototype human mitochondrial DNA standard, SRM 2392.

Several interlaboratory tests are ongoing to verify the suitability of NIST-developed materials for use as national and international standards. Such tests include specific DNA sequences to assure consistent laboratory performance in sequencing human DNA for identification purposes. A synthetic challenge sequence for maintaining quality assurance with automated DNA sequencers has also developed. The development of such essential reference materials and expanding the technical expertise to produce them are key elements in successfully maintaining U.S. leadership in genomic technology.

Recent studies on methods for detecting **single base mutations** have focused on the use of DNA in diagnostic measurements for genetic disease detection and for rapid detection of microbial species. Fruitful research with one biotechnology company through a cooperative research and development agreement (CRADA) has led to development of rapid capillary electrophoretic (CE) methods for mutation detection. Additional research is centered on developing procedures for Single-Strand Conformation Polymorphism detection by CE. This method allows detection of very small changes in DNA. Use of newly-discovered enzymes to elucidate DNA oligonucleotide structure is also becoming a focus of the group. CRADA work with another biotechnology company led to the filing of a joint patent in the field of rapid DNA **amplification and detection of large fragments of DNA**.

Long-range research in the Group includes the development of methods for detecting and quantifying **DNA damage and repair**, processes that are critical to understanding the genesis of cancer. Methods have been developed to characterize DNA damage on a molecular scale at levels approaching one base per million using GC/MS techniques, and recently these methods have been used to study the kinetics and specificity of DNA repair by specific enzymes. Additional studies are concentrated on the study of apoptosis, or programmed cell death, as well as detection of cellular responses to radiation.

Finally, exciting basic research in the Group in molecular biology has concentrated on the cloning, expression, and purification of an important **DNA polymerase** which could be used to more accurately sequence problematic DNA sequences containing high Guanine-to-Cytosine ratios.

The **Bioprocess Engineering Group** is focused on the development of measurement methods, databases, and generic technologies related to the use of biomolecules and biomaterials in manufacturing. In FY 1996, measurement methods and data have been developed in the protein **electro/optical probing** project which will lead to improved understanding of intra- and inter-protein electron transfer processes. This understanding aids industrial biocatalyst development by allowing for 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. In the **enzyme-catalyzed reaction thermodynamics** project, chromatography and micro calorimetry measurements have been combined with chemical equilibrium analysis to develop thermodynamic for three industrially important biotransformations: 1) conversion of xylitol to D-xylose by L-iditol 2-dehydrogenase, 2) the essentially stereospecific conversion of tetralin(aq) to (R)-1-tetralol(aq) by cytochrome P-450 monooxygenase, and 3) the hydrolysis of α-, β-, and γ-cyclodextrin by combination of enzymes (amyloglucosidase + α-amylase or amyloglucosidase + cyclodextrin glucanotransferase). For the cyclodextrin studies, efforts are now underway to measure equilibrium constants for cyclization reactions such as α-cyclodextrin(aq) + D-glucose(aq) = maltoheptaose(aq). In the **preparative DNA separations** project, electrochromatographic/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. In FY 1996, two patents have been filed on this topic. New projects in **enzyme characterization** by **nitrogen-15 NMR spectroscopy** and by **X-ray diffraction of protein crystals** have been initiated. These techniques will be used to address focused, industrially-important biotransformation problems in FY 1997.

The **Structural Biology Group** is located at the **Center for Advanced Research in Biotechnology** (CARB) on the University of Maryland Shady Grove Campus about four miles from NIST. CARB is a joint collaboration of the Biotechnology Division and the University of Maryland Biotechnology Institute. Scientists at CARB develop and apply measurement methods, databases, and state-of-the-art modeling methods to advance the understanding of protein structure/function relationships. X-ray crystallographic and high resolution Nuclear Magnetic Resonance (NMR) methods are used to measure the structures of prototypical proteins, enzymes, enzyme-substrate complexes, and other macromolecular systems. These three-dimensional structures not only provide a database for the development of theoretical models for macromolecular interactions, but also guide the Group's efforts in protein engineering and design using state-of-the-art methods in molecular biology. Combining structural and theoretical studies with kinetic and thermodynamic information from complementary physical measurement methods including calorimetry and spectroscopy is essential for understanding structure/function relationships such as thermodynamic stability, protein folding pathways, protein ligand interactions, and enzymatic reaction pathways.

Expansion of the CARB facility is currently underway, with construction estimated to be complete in the summer of 1997. The new annex to the existing building will provide an additional 28,000 square feet of research space. This expansion will alleviate the pressing shortage of adequate research space needed to sustain CARB's existing programs, and also to accommodate future directions of the Structural Biology Group. The group has added one senior investigator, Dr. James Stivers, who is initiating experimental studies in the area of mechanistic enzymology. A search for a second addition to the senior staff in the area of high resolution structure determination by multidimensional NMR spectroscopy is underway.

The highlights of the Structural Biology Group's research include diverse activities all centered around the elucidation of how protein structure dictates function. The development of experimental **measurement methods** to determine the cooperative energetics and pathways of folding soluble and **membrane embedded proteins** continues to be an important component of these activities. Recent results on the functional reassembly of inactive fragments of a prototypical seven-transmembrane helix, **G-protein coupled receptor** have identified several sets of complementing polypeptide chains which suggest pathways for the assembly of the multiple protein folding domains of the receptor. A number of allosterically-controled protein systems are under investigation. The mechanism for the activation of transcription in *E. coli* by **cAMP receptor protein** illustrates how biophysical techniques such as calorimetry are being used to investigate this fundamental activity of this class of protein.

Novel algorithms in **computational chemistry** have been developed in the Structural Biology Group for predicting protein-ligand conformations and energies. Future efforts are focused on modeling solvent effects, and on examining inhibitors of HIV protease. Database activities include continued increase in the data and the capabilities of the World Wide Web version of the **NIST/NASA/CARB Biological Macromolecule Crystallization Database**.

The Structural Biology Group continues to expand its interactions with industry. Two new CRADAs were established, one with MedImmune to undertake a thermodynamic characterization of monoclonal IgM using calorimetric methods and the other with Genetics Institute, Inc. to explore biophysical and crystallographic attributes of thioredoxin fusion peptides and proteins. One of the Group's staff, Diane Hancock, spent a sabbatical year as a NIST Industry Fellow with DuPont Merck Pharmaceuticals, Inc. working in their NMR group. She was the first Biotechnology Division staff member to take advantage of this new program.

The **Biomolecular Materials Group** was formed in October, 1996. This group grew out of the Biosensors Technology Group, and still retains interest in biosensors. However, it was appreciated that the most critical issues in the field of biosensors today are also critical issues in the areas of diagnostics, novel materials, bioelectronics, biocatalysis, and other outgrowths of Biotechnology: the controlled chemical manipulation, the functional activity, and the structural characterization of biological and biomimetic molecules and surfaces. To this end, the Biomolecular Materials Group has several key programmatic thrusts. One area is research on **hybrid bilayer membranes**, which are rugged models of cell membranes that serve as matrices for reconstitution of active membrane proteins. These supported model membranes are being used for structural studies of membranes and membrane proteins using vibrational spectroscopies and neutron reflectivity. They are also being used in practical applications such as pharmaceutical screening by a CRADA collaborator, SmithKline Beecham, and are being explored as matrices for transmembrane proteins such as redox enzymes, cell surface receptor proteins, and ion pores.

The structure and function of the light sensitive membrane protein, **bacteriorhodopsin**, is also of interest in the group. Work in this area is focusing on the reconstituion of chromophore analogues of bacteriorhodopsin. These studies will add to understanding of this membrane protein's structure and mechanism of activity as well as further the development of its applications in optical displays and sensors. Alpha toxin of *Staphlococcus aureus* is another membrane protein under study in the Biomolecular Materials Group. In addition to understanding the mechanism of action of this important class of **pore-forming toxins**, the development of strategies for using such molecules as the basis of sensor and separation devices is being explored.

Meeting the future needs of the biotechnology industry will require the strengthening of existing Division 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 be a high priority in FY 1997 because of the rapid growth of industrial applications in this area. At CARB, upgrading the existing X-ray crystallography facility and establishing a state-of-the-art NMR facility in collaboration with the University of Maryland are high priorities. Included in Division plans are the continued expansion of programs in biomolecular materials, which reflects and 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, expanded emphasis will be placed on the development of modeling which 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 bioprocess technology, with an emphasis on measurements and data to help industry solve generic problems that limit technology development. Also planned for the Division in FY 1997 will be an expanded effort in the area of bioinformatics, which includes 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 our 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:
Dr. Gary Gilliland was awarded the Department of Commerce's Silver Medal for his leadership of the unique joint program between NIST and University of Maryland Biotechnology Institute at CARB and for his contributions to the NIST biotechnology program through his world-renowned research in protein crystallography.

B. Selected Technical Reports

1. *Detection of p53 Point Mutations by Capillary Electrophoresis*

D.H. Atha, H.M. Wenz (Perkin Elmer, Inc.), H. Morehead, J. Tian (Geo-Centers, Inc.) and C. O'Connell.

Objective: To develop capillary electrophoresis (CE) as an alternative analytical tool for the detection of DNA mutations in the p53 gene. Test and optimize the detection of single strand conformational polymorphism (SSCP) by CE as a means to develop sensitive DNA diagnostic methods.

Problem: There is an increasing need in DNA diagnostics for more efficient methods of detecting mutations associated with genetic diseases. Most of the established methods for mutation detection use amplification technologies and slab gel electrophoresis. Continuing improvements in the sensitivity and automation of CE, including improved capillary systems and separation conditions, make CE an increasingly attractive alternative for the detection of these mutations. However, methods involving CE need to be tested and optimized for each diagnostic system. The p53 tumor suppressor gene was selected to develop these measurement techniques because of its wide-spread use by industrial and academic scientists in the development of diagnostic tests for various cancers and because of the extensive information available for this system. The p53 tumor suppressor gene codes for a phosphoprotein which has cancer inhibiting functions. Cancer development in humans very often involves loss of wild-type p53 activity and its control over cell growth.

Approach: A 2.2 kilobase region of p53 containing exons 5-9 was cloned from seven cell lines reported to contain common p53 mutations. We analyzed the seven cell line p53 mutations by single strand conformational polymorphism capillary electrophoresis (CE-SSCP) and have compared these measurements to previous measurements obtained by slab gel electrophoresis (SG-SSCP). Five of seven cell line mutations are single base substitutions; one contains two base changes, and the seventh cell line has a single base deletion. PCR primers were used for amplification of specific exons for mutation detection. 5' Primers were labeled with FAM (5-carboxyfluorescein) and 3' primers were labeled with JOE (2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein). CE-SSCP was performed using two different CE systems.

Results and Future Plans: Although the relative shifts in migration times for the p53 mutations could be successfully detected by either slab gel or capillary electrophoresis, the measurements were about tenfold faster and more automated with CE. The CE-SSCP measurements were performed at temperatures ranging from 16 °C to 50 °C. For mutations measured at ambient temperature (25 °C), characteristic shifts in direction and magnitude were observed in the migration times of both strands relative to the wild type. This demonstrated the ability of CE at ambient temperature to resolve these mutations. However, the magnitude and direction of shifts in migration time varied with temperature in a discrete pattern for each mutation. This demonstrated that extended temperature control will be an important advantage in resolving single point mutations by CE-SSCP. In addition, by using the higher resolving CE, intra-strand hybridized structures could be observed as the temperature was scanned. The ability to discriminate between mutations that produce similar shifts in migration time but produce unique fingerprints due to minor isoforms would be useful in a diagnostic setting. Further experiments are underway to understand the structural nature of these fingerprint peaks and their interrelationship to SSCP separations.

In FY 1997, additional p53 mutations will be examined by CE. The comparison of CE-SSCP data produced by a wide range of point mutations will provide a better understanding of the effect of structure on the sensitivity of SSCP. We expect that these results will be of assistance to the diagnostic community to evaluate potential uses for CE-SSCP in the detection of genetic mutations.

2. *Long Polymerase Chain Reaction (PCR) for Variable Number of Tandem Repeats (VNTR) Analysis*

C.D. O'Connell, K.L. Richie, and D.J. Reeder

Objective: To apply long polymerase chain reaction (PCR) technologies to the analysis of variable number of tandem repeats (VNTRs) used in human identification by constructing primers specific for the human loci most commonly examined by restriction fragment length polymorphism (RFLP) analysis and present in the forensic database CODIS.

Problem: The occurrence of VNTRs present in the human genome is commonly used for human identification. The shorter repeat units are examined by PCR amplification technologies, whereas the longer units are examined by RFLP analysis. RFLP analysis is labor-intensive and time consuming, yet data obtained from these longer repeat units are statistically more significant for human identification than those obtained from PCR-amplified short repeats. The ability to convert the RFLP assay system to a PCR-based system will result in substantial savings of both time and labor to the human identity community.

Approach: The human identity community has used RFLP analysis of long repeat units present in human DNA for nearly ten years. A number of data banks with data obtained from thousands of individuals have been constructed and are used by this community to attach statistical relevance to individual DNA profiles. The availability of these data has resulted in the acceptance of DNA evidence in the courtroom. Shorter repeat units have started to gain acceptance by the human identity community as they are easier to assay using PCR amplification technologies. A typical short tandem repeat (STR) DNA profile can be completed in one day, whereas an RFLP profile takes a minimum of two-three weeks. The STRs used for human identification are not as polymorphic as the long repeats. Therefore, more genomic loci are used (9-12) to obtain the same level of statistical significance as four RFLP loci. With the release of several assay systems which allow one to PCR amplify genomic DNA fragments of 25 kilobase (kb) or more, we sought to determine whether we could amplify the RFLP loci from forensic samples, preferably human blood and bloodstains. PCR primer pairs specific for human genes are available to amplify genomic DNA targets of 4.8 kb, 9.3 kb, and 15 kb. The majority of RFLP alleles present in the human population are smaller than 9.3 kb. We therefore decided to use these primer pairs to determine feasibility of amplifying DNA isolated from human bloods, including aged blood samples, using these primer pairs. We decided to use DNA isolation procedures that are widely accepted by the forensic community for these experiments. Our ultimate goal, if feasibility was established, was to use primer pairs specific for the RFLP loci to amplify DNA isolated from forensic samples.

Results and Future Plans: DNAs were isolated from both freshly obtained human blood as well as aged blood samples which had been previously examined by RFLP analysis. We successfully determined PCR conditions to amplify both 4.8 and 9.3 kb DNA fragments specific for the human tissue plasminogen activator (TPA) gene. Although the amplification from aged bloods was less robust, PCR conditions were established which allowed us to visualize easily the PCR products by agarose gel electrophoresis and ethidium bromide staining. This is a widely used molecular biology technique to analyze DNA. Having established feasibility of amplifying forensic samples in the size ranges common to RFLP loci, we then determined whether the Long PCR enzymes and reagents could amplify a DNA region containing repeat sequences. The blood samples were PCR amplified with primer pairs specific for a longer STR, *D1S80*, using both standard PCR enzymes and reagents and a Long PCR system. Identical results were obtained with the two systems, suggesting that primer pairs specific for RFLP loci would also result in amplification products of the correct size. A CRADA agreement was established with Life Technologies, Inc. (LTI) to continue this project. LTI has obtained DNA sequence information and primer pairs specific for two of the RFLP loci commonly used for human identification. Using these primer pairs, we have demonstrated that these loci can be PCR-amplified. Research is underway to optimize amplification conditions and to obtain primer pairs for the other RFLP loci used for human identification.

3. *Kinetics of Excision of Purine Lesions from DNA by Fpg Protein*

A. Karakaya (University of Ankara, Ankara, Turkey), P. Jaruga (Medical Academy, Bydgoszcz, Poland), and ***M. Dizdaroglu***

Objective: To determine the kinetics of excision of modified purine bases by Fpg protein from oxidatively damaged DNA using different DNA substrates.

Problem: *E. coli* Fpg protein is a DNA repair enzyme which removes modified purine bases from DNA as a DNA *N*-glycosylase by hydrolyzing the glycosidic bond between the sugar moiety and modified bases. This is a major DNA repair enzyme in *E. coli,* and its counterparts exist in eukaryotic cells including human cells. A major function of this enzyme is to protect cells from premutagenic purine lesions that are produced in DNA by the action of free radicals or other oxidizing agents. However, the kinetics of the reaction by which purine lesions are excised from oxidatively damaged DNA by Fpg protein has not been reported. Further, it was not known whether the excision kinetics depends on the nature of the DNA substrate.

Approach: Oxidative damage generates a multitude of modified bases in DNA. An accurate measurement of these lesions is achieved by the use of the GC/MS technique. Because of its ability to simultaneously measure a large number of DNA lesions, this technique permits to study the substrate specificity of a DNA repair enzyme toward a multitude of DNA base lesions, and to determine kinetics of removal of the lesions from DNA. We used this unique approach to determine the kinetics of excision of three purine lesions from various DNA substrates. These lesions were 8-hydroxyguanine (derived from guanine), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (derived from guanine) and 4,6-diamino-5-formamidopyrimidine (derived from adenine).

Results and Future Plans: DNA substrates were prepared by γ-irradiation under oxic or anoxic conditions and by treatment with H_2O_2 plus metal ions. These substrates were incubated either with the active enzyme (Fpg protein), with the heat-inactivated enzyme, or without the enzyme. DNA was ethanol-precipitated. Supernatant fractions after derivatization, and DNA pellets after hydrolysis and derivatization, were analyzed by GC/MS. The results revealed efficient excision of 8-hydroxyguanine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine, and 4,6-diamino-5-formamidopyrimidine from all three DNA substrates. Thirteen other modified bases found in DNA substrates were not removed by the enzyme. Excision was measured as a function of enzyme concentration, incubation time, substrate concentration, and incubation temperature. Kinetic parameters such as maximum velocity, catalytic constant, Michaelis constant, specificity constant, and activation energies of excision were determined. Kinetics of excision of modified purine bases from the three damaged DNA substrates varied significantly even though each substrate contained similar levels of oxidative damage, indicating a strong dependence of kinetics on the nature of the DNA substrate. A comparison of the specificity constants for each DNA substrate revealed a comparable preference of the enzyme for the three purine lesions. Recent studies of Fpg protein have focused on 8-hydroxyguanine as the physiological substrate for this enzyme. Our results indicate that 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 4,6-diamino-5-formamidopyrimidine also may be important physiological substrates for this enzyme.

This work will be extended to other DNA repair enzymes such as *E. coli* Nth protein, *E. coli* endonuclease VIII, yeast Ogg1 protein (counterpart of Fpg protein in yeast), yeast Nth protein, and human counterpart of Fpg protein. The substrate specificities of these enzymes will be investigated and excision kinetics of modified bases will be determined. These studies will contribute to understanding the mechanisms of action of DNA repair enzymes in terms of individual pyrimidine and purine lesions in DNA.

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4. *Determination of Nuclear and Mitochondrial DNA Damage from Chemical and Physical Agents Using Cytogenetic and Sequencing Techniques*

B.C. Levin, Z. Nackerdien, H. Cheng (GeoCenters), J. Chen, V. Cetolla, and D.J. Reeder

Objective: To develop the capability to use cytogenetic assays such as the single cell gel assay (comet assay) and the micro-nucleus test as well as direct sequencing techniques to examine the DNA damage (double and single strand breakage, base pair mutations) that occurs in human cells following exposure to chemical and physical agents.

Problem: DNA damage can lead to diseases such as cancer, and if damage causes mutations in germ cells, these adverse effects can be transferred to future generations. Therefore, it is important to be able to detect the damage that has occurred. If all the cells have the same inherited mutation, this difference can be detected by sequencing the area containing the change. The problem arises when the exposure of the DNA to a physical or chemical agent affects some of the cells, but not others, or affects the DNA in a random way (*i.e.*, some mismatching of base pairs occurs, but there is no specific hot spot). We are developing an approach to delineate genetic changes that occur following adverse environmental exposures.

Approach: *The Single Cell Gel Assay*: This assay is able to detect single-strand and double-strand DNA breaks in individual eukaryotic cells. The assay requires small numbers of cells (<20,000 per sample), can detect DNA damage from low levels of toxic or physical insults, and is rapid, simple and efficient. In this assay, cell membranes are lysed, and the cells are placed in an electric field. If the DNA has single or double-strand breaks, it will flow out of the cells and move toward the anode causing the cell and its DNA to resemble a comet. The more DNA released from the cell, the greater the amount of DNA damage. A computerized imaging system is used to score and measure the cometary patterns.

Micronucleus Test: The cytokinesis-blocked micronucleus test is also used to detect chromosome breakage from chemical and physical insults. The main advantages of this test are its ease of performance and quick results. Like the single cell gel assay, the micronucleus test can be used for examination of damage following both *in vitro* and *in vivo* exposures. However, neither can detect less drastic aberrations, such as translocations, inversions, transitions, transversions, insertions, or deletions. They also cannot distinguish between the types of insults, e.g., radiation-induced effects versus other nonspecific damage.

PCR and Sequencing: Amplification and sequencing of the damaged areas of the DNA allow one to detect inversions, transitions, transversions, insertions or deletions. The problem that needs to be solved is the separation of the damaged DNA from the undamaged DNA so that the damage is not overshadowed by the undamaged DNA.

Results and Future Plans: The Single Cell Gel Assay has been tested with human lymphoblastoid, EBV transformed lymphocyte, and HL-60 leukemia tissue culture cells. We have examined the effects of ^{60}Co-γ-radiation, hydrogen peroxide, and azido-thymidine (AZT), all of which cause DNA strand breakage and produce cometary patterns in the assay. The micronucleus test is still in the preliminary stages of development. PCR and sequencing are routinely done in our laboratory especially with human mitochondrial

DNA. We plan to examine cloned DNA as a means to separate the damaged DNA from the undamaged DNA.

5. *Cloning, Expression, Purification of DNA Polymerase I for use in DNA Sequencing*

Prasad Reddy and Madhavi Reddy (Maulana Azad Medical College, New Delhi, India)

Objective: To isolate a DNA polymerase I from mycobacteria that can accurately and efficiently edit and polymerize its own chromosomal DNA, and to explore the utility of the enzyme for sequencing DNA with high guanine:cytosine base pair (G:C) content.

Problem: The genome of mycobacterial species contains a high ratio of about 70% G:C. Consequently, deciphering the nucleotide sequence of the genome and of the individual genes of this microbe(s) is extremely difficult. While sequencing DNA molecules from mycobacteria on the Applied Biosystems 373 instrument, we observed sequence-dependent patterns and artifacts in the signal strength for the dideoxynucleotides cytosine and guanine. In many sequences where the GC dinucleotide occurs, the C signal is extremely small when the cycle sequencing is carried out with Taq CS DNA polymerase. This sequence artifact is eliminated if the cycle sequencing is carried out with Taq FS polymerase. However, Taq FS is not a perfect enzyme for sequencing DNA. In cases where the CG or AG dinucleotides occur, the signal strength for G is very small when the cycle sequencing is carried out with Taq FS. This causes the sequence calling software to insert an N or nothing in the worst case. The magnitude of the problem increases in DNA with high G:C content. So at this stage there appears to be no perfect polymerase for sequencing high G:C content DNA molecules.

Approach: In order to address the problems associated with the sequencing of G:C rich genomes, we have chosen to clone the gene for DNA polymerase I(polA) from *Mycobacterium thermoresistibile*. The rationale is that the DNA polymerase from this microbe would be active at high temperature which is a requirement for cycle sequencing. Also, the polymerase from a microbe with high G:C content should be able to decipher the DNA sequence of molecules with high G:C content. The gene polA would be isolated and cloned into the pRE expression vector to overproduce the enzyme. The purified enzyme would be tested for its ability to sequence mycobacterial DNA.

Results and Future Plans: The polA gene of *Mycobacterium* species was cloned by utilizing the standard molecular biology techniques. The gene was sequenced, and cloned into the pRE expression vector for overproducing the enzyme. Full length DNA polymerase I of relative molecular mass, M_r, 100,000, was overproduced to about 20% of the total cell protein. Two truncated products, $M_r = 70,000$ and $M_r = 55,000$, were also produced. The full length protein was found to be an aggregate (inclusion bodies). The truncated products were obtained as soluble polypeptides. DNA polymerase I possess three catalytic functions, namely 5' to 3' DNA polymerization activity, 5' to 3' exonuclease activity and 3' to 5' exonuclease proofreading activity. Analysis of the intact and the truncated polypeptides for these activities revealed the following results. The full length polymerase I in the inclusion bodies had no enzyme activity while the polypeptides with $M_r = 70,000$ possessed 5' to 3' polymerization activity. The polypeptide with $M_r = 55,000$ had no 5' to 3' polymerization activity.

In order to fold polymerase I into an active conformation from the inclusion bodies, GroEL and GroES protein folding chaperones were overproduced and purified. The polymerase I will be solubilized in urea and the folding of the enzyme into an active conformation will be achieved. The DNA sequencing will be carried out with this polymerase I and compared with the sequence generated with the commercially available polymerases.

6. *Electro/Optical Probing of Proteins on Modified Metal Electrodes*

***A.K. Gaigalas**, V. Reipa (UCLA), L. Wong (UCLA), W. Yap, G. Niaura (Vilnius State University, Vilnius, Lithuania), A. Roitberg (Geo-Centers), V.L. Vilker*

Objective: To measure the structural changes occurring in proteins during redox processes on modified electrodes; to measure electron transfer rates associated with these redox processes; and to develop models for electron transfer in proteins which explain and correlate the experimental results.

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 lack of inexpensive and reliable sources of reducing power. This work will provide a rational scheme for the design and implementation of multiprotein redox enzyme systems such as those found in industrial biocatalysts, and will allow for development of sources of reducing power based on modified electrodes.

Approach: Electrochemical measurements such as linear sweep voltammetry (LSV), surface enhanced Raman spectroscopy (SERS), electroreflectance spectroscopy (ER), and spectroellipsometry (SE) are being used to characterize electrode surfaces and to measure electron transfer (ET) in several redox proteins [e.g. putidaredoxin (iron-sulfur redox center), azurin (copper redox center), cytochrome c (heme redox center)]. LSV provides a diagnostic technique for determining general suitability of specific modified electrode/protein combinations. SERS provides molecular-level information about structural changes that proteins undergo during changes in oxidation state, as well as structural signatures of electrode-modifying layers. ER provides means to determine ET kinetics and to evaluate the importance of specific protein parameters controlling these rates. In-situ characterization of the electrode/solution interface is rendered by SE in conjunction with electrochemical techniques.

Results and Future Plans: SERS has been used to identify metal site vibrations in cytochrome c, putidaredoxin (Pdx) and azurin. These are being used to monitor redox processes from proteins on adsorbed layers. ER has been used to measure the redox reaction rate for Pdx on bare or bekanamycin-modified (BK) silver electrodes. The electron turnover rates were determined using electroreflectance (ER) and voltammetry (LSV). The measurements were analyzed with two kinetic models: i) strongly adsorbed protein layer, and ii) freely diffusing protein. For the adsorbed layer model, the rates obtained with ER were almost two orders of magnitude greater than the rates obtained with LSV. For the freely diffusing model, the rates determined by the two methods were similar suggesting that this kinetic model is more appropriate. Modification of the Ag electrode with bekanamycin resulted in a negative 0.1 V shift of the Pdx formal potential, but showed only a small increase in the observed redox reaction rates. The measured rate constants were compared to the predictions of Marcus theory of electron transfer (ET). Calculation of the temperature dependence of the ER response is shown to depend on the nuclear reorganization energy and the electron coupling matrix element of the redox protein. Similar measurements and analyses are being done for azurin adsorbed on modified gold electrodes. Recently, ER has been combined with NIST's state-of-the-art synchrotron ultra-violet light source (SURF II) in order to study the effects of electric fields on the structure of adsorbed proteins as well as the characteristics of electron transfer between the protein metal center and the electrode. A computational effort has been initiated in order to build mechanistic insights from these experimental results, along with structural information from NMR and crystallography studies.

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7. *Thermodynamics of Enzyme-Catalyzed Reactions*

R.N. Goldberg, Y.B. Tewari, M.P. Mayhew (Geo-Centers), and V.L. Vilker

Objective: To develop data and models that are required for the use of enzymes in industrial processes.

Problem: A 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: The accomplishment of this research relies upon a combination of techniques: chromatography, microcalorimetry, thermodynamic modeling, and effective utilization of results in the literature. This combination allows for checks on the consistency of the measurements and for the development of suitable thermodynamic models. This frequently allows one to make reliable extrapolations of the data outside of the region in which actual measurements have been performed. 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: Several studies have been completed and/or initiated. In one study, the enzyme L-iditol 2-dehydrogenase was used to catalyze a series of (NAD + NADH) coupled reactions. One of these reactions involves the conversion of xylitol to D-xylulose, a key step in the xylose assimilation pathway which is central to the conversion of hemicellulosic materials to ethanol.

The essentially stereospecific conversion: tetralin(aq) + NADH(aq) + O_2(aq) = (*R*)-1-tetralol(aq) + NAD(aq) + H_2O(l), which is catalyzed by camphor (cytochrome P-450) 5-monooxygenase, has been the subject of both a thermodynamic and kinetic investigation. Approximate values of a Michaelis constant K_m, limiting rate V_{max}, and catalytic constant k_{cat} were obtained for this reaction under a specified set of conditions. Values of the standard molar Gibbs energies and standard molar enthalpies for this reaction were obtained using a thermochemical cycle calculation. It was found that this reaction is irreversible in the direction of complete formation of 1-tetralol from tetralin, and that dimethyl sulfoxide, methanol, and p-dioxane serve as reaction accelerators.

Calorimetric measurements have been performed on the hydrolysis reactions of α-, β-, and γ-cyclodextrin. These reactions were catalyzed using a combination of enzymes, namely (amyloglucosidase + α-amylase) or (amyloglucosidase + cyclodextrin glucanotransferase). We are now attempting to measure equilibrium constants for cyclization reactions such as α-cyclodextrin(aq) + D-glucose(aq) = maltoheptaose(aq).

The last of a series of reviews on the thermodynamics of enzyme-catalyzed reactions has been published.

Current plans call for studies of reactions leading to the formation of aromatic amino acids and for studies on the chorismate/shikimate metabolic pathways.

Publications:

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Goldberg, R.N. and Tewari, Y.B., "**Thermodynamics of Enzyme-Catalyzed Reactions: Part 5. Isomerases and Ligases,**" J. Phys. Chem. Ref. Data, 24, 1765-1801 (1995).

Tewari, Y.B., Schantz, M.M., Rekharsky, M.V., and Goldberg, R.N., "**Thermodynamics of the Hydrolysis of 3,4,5-trihydroxybenzoic Acid Propyl Ester (n-propylgallate) to 3,4,5-trihydroxybenzoic Acid (gallic acid) and propan-1-ol in Aqueous Media and in Toluene,**" J. Chem. Thermodyn. 28, 171-185 (1996).

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Tewari, Y.B. and Goldberg, R.N., "**Thermodynamics of reactions catalyzed by L-iditol 2-dehydrogenase:**

the xylose assimilation pathway," J. Chem. Thermodyn. (in press).

8. *Preparative Separations of Different DNA Physical Forms Using Electrochromatography*

K.D. Cole

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 has been done in collaboration with the company Life Technologies, Inc. (LTI). LTI has provided a crude cellular extract which contained supercoiled circular DNA, nicked circular plasmid DNA (relaxed), linear genomic DNA (from host bacteria), RNA , and other cellular constituents. We have also developed improved equipment for electrochromatography.

Results and Future Plans: Electrochromatography was effective at separating the supercoiled plasmid, relaxed circular plasmid, and linear genomic DNA. The separation of different physical forms of DNA was governed by the electric field strength, the flow rate, and the porosity of the chromatography packing material. Plasmid DNA of different relative molecular mass was size-fractionated by varying the electrical field strength. At a given electrical field strength and flow rate, M_r DNA plasmid fragments were not as strongly retained as higher M_r plasmid fragments. By selecting a specific set of conditions (packing material, flow rate, and electrical field strength) the relative molecular mass of DNA fragments retained by the column was adjusted. Improved electrochromatography fittings were developed that allow uniform and reliable electrical fields to be applied to chromatography columns. Pulsing the applied electric field was shown to be a powerful variable in the separation of different physical forms of plasmid DNA. The time scale of electric field pulsing was important in determining the retention of the different forms of DNA. In the future, we will examine the behavior of very large DNA (such as bacterial artificial chromosomes) and small DNA (oligonucleotides). Preparative separations of both large and small DNA molecules are necessary for research and production needs of industry.

Publications:

Nivinskas, H. and Cole, K.D., **"Environmentally Benign Staining Procedure for Electrophoretic Gels Using Coomassie Brilliant Blue,"** BioTechniques 20, 380-383 (1996).

Cole, K.D. and Cabezas, Jr., H., **"Improved Preparative Electro-chromatography Column Design,"** Journal of Chromatography A (in press).

9. *Enzyme Characterization I: Nitrogen-15 NMR Spectroscopy of Metalloproteins*

B. Coxon, J. Edwards, M. Holden (Geo-Centers, Inc.), and N. Sari (Bogazici University, Istanbul, Turkey).

Objective: To investigate the structure and dynamics of the oxidized form of putidaredoxin (Pdx), an electron transfer protein that is a component of a ternary cytochrome P450 enzyme system which promotes the regio- and stereo-specific hydroxylation of hydrocarbons.

Problem: The iron-sulfur cluster in Pdx plays an important role in the electron transfer processes that control the catalytic activity of cytochrome P450. However, of the 106 amino acid residues in Pdx, about 20 are not well characterized by proton NMR spectroscopy because of a paramagnetic broadening that is worse than that in many metalloproteins. As a result, there is little firm structural information available for the atoms in the immediate vicinity (<8 Å) of the iron-sulfur cluster.

Approach: In close proximity to the paramagnetic center, where nuclear relaxation is dominated by electron→nuclear dipole-dipole interaction, NMR linewidth is proportional to the square of the magnetogyric ratio (γ) of the NMR active nucleus. Because $\gamma_{N\text{-}15}$ is about 10 times smaller than $\gamma_{H\text{-}1}$, nitrogen-15 NMR linewidths may be expected to be up to 100 times smaller than proton NMR linewidths for nuclei in the paramagnetic zone. We have therefore investigated the structure of Pdx-^{15}N by direct observation of nitrogen-15 nuclei at their own resonant frequency (40.55 MHz).

Results and Future Plans: Samples of uniformly labeled Pdx-^{15}N have been prepared by insertion of suitable plasmids into *E. Coli*, followed by temperature controlled growth on a medium which contained ammonium-^{15}N chloride as a nitrogen source. Protein preparations examined by high-resolution two-dimensional gel electrophoresis had mole fraction purities greater than 97%. The structures of Pdx and Pdx^{15}N have been studied by homo- and hetero-nuclear NMR methods, including identification of ^{15}N resonance types by one- and two-dimensional DEPT (distortionless enhancement by polarization transfer) spectrum editing, and measurements of ^{15}N chemical shifts, coupling constants, nuclear Overhauser effects, and spin-lattice relaxation times. Direct detection of ^{15}N NMR spectra has revealed a number of ^{15}N resonances which have not been found in previous ^{1}H detected NMR studies, including 17 paramagnetically broadened signals from ^{15}N nuclei which are structurally close to the iron-sulfur cluster of the protein, four sharp ^{15}N resonances for the proline residues, three lysine sidechain ^{15}N resonances, and several other sidechain resonances.

Because most of the residues that are missing from the ^{1}H detected data reported previously are amino acid residues without sidechain nitrogen atoms, we can conclude that the majority of our 17 broad ^{15}N resonances originate from the *backbone* ^{15}N atoms of residues that are close to the paramagnetic center. By use of directly observed ^{15}N NMR spectra and the inversion recovery method, we have measured a set of ^{15}N spin lattice relaxation times (T_1) from those resonances that are clearly separated and/or are sufficiently intense in the 1D ^{15}N NMR spectra, including data from a number of broadened and unbroadened signals that originate from backbone ^{15}N atoms both within and without the paramagnetic zone of the protein, respectively.

By analysis of the ^{15}N T_1 values, we have calculated reduced distances of ^{15}N nuclei from the iron-sulfur cluster, for a number of such nuclei that are within ~4 Å - 7 Å of this cluster, this being the valid range of distances for calculations based on the assumption of relaxation by a dominant electron→nuclear dipole-dipole interaction. Ranked by size, the resulting reduced distances are in reasonable agreement with the range of distances calculated as averages of the distances in 12 closely related structures of Pdx which have been defined by homonuclear ^{1}H NMR methods. However, a detailed, one-to-one comparison of the latter reduced distances with those calculated from the ^{15}N T_1 values will require specific assignments of the paramagnetically broadened ^{15}N resonances, which are not available at the present time.

In the future, we plan to investigate two approaches to the ^{15}N assignments which involve selective ^{15}N labeling and mutant proteins. For paramagnetic proteins that do not readily exhibit ^{1}H-^{1}H NOEs within the paramagnetic zone, the present results emphasize the utility of determination of distances within this zone from ^{15}N T_1 data.

Publications:

Edwards, J. and Holden, M., **"Purification and Electro-phoretic Characterization of the Electron Transport Protein Plastocyanin from Spinach,"** Appl. Theoret. Electrophoresis (accepted).

10. *Enzyme Characterization II: Diffraction-Based Structures and Crystal Growth Processes*

***D.T. Gallagher**, G.L. Gilliland, and M. Holden (Geo-Centers, Inc.)*

Objective: To understand the process of protein crystal growth and make diffraction measurements leading to useful structural models.

Problem: Protein and DNA structures are increasingly important to the pharmaceutical and biotechnology industries. Obtaining these structural data relies on diffraction measurements from crystals whose growth is poorly understood and is often the bottleneck in structure determination.

Approach: Crystal growth of several enzymes is under systematic study. Subtilisin, the widely used commercial and industrial protease, has been analyzed and compared in several polymorphs. In general, the processes governing the differential effects of temperature, pH, ionic strength, and other parameters on crystal growth will be analyzed at the molecular level. For example, the morphology of subtilisin crystals is dependent on ambient growth conditions, and these effects are due to the chemistry of crystal contact formation at protein-protein interfaces in growing crystals. Diffraction measurements and structural models will be pursued for all crystals obtained.

Results and Future Plans: Three polymorphs of Subtilisin BPN' have been refined at high resolution (1.8 Å or better) and compared. One of these is the highest- resolution structure yet reported for this industrially- important enzyme. The comparison yields several important insights into the crystal growth process. For example, analysis of hydration in the three crystal forms shows that crystal contacts tend to form by overlapping bound water sites in a condensation-like reaction. Further studies will focus on how the shapes of growing crystals are affected by modulations of chemical conditions. Under appropriate conditions these observations provide information on how the strengths of crystal contacts depend on their chemical environment. Studies are also planned to examine proteins from multiprotein enzyme systems before and after binding with electron transfer partner proteins in order to determine binding sites and electron transfer pathways.

Publications:

Gallagher, T., Oliver, J., Bott, R., Betzel, C., and Gilliland, G.L., "**The Structure of Subtilisin BPN' at 1.6 Angstroms Resolution and Three High-Resolution Crystal Forms Compared,**" Acta Crystallographica, Section D (in press).

11. *Theory of Non-Covalent Protein-Ligand Binding*

***M.K. Gilson**, M.S. Head, and J.A. Given (CARB/ University of Maryland)*

Objective: To understand the physical chemistry of specific, noncovalent binding of small molecules by proteins, and to incorporate this understanding into predictive computer models.

Problem: The specific binding of small molecules by proteins is of central importance in enzyme function and drug action. However, the mechanisms of this phenomenon are not fully understood. Predictive models for binding would be of great practical value in applications such as altering the substrate specificity of enzymes, and structure-based drug design.

Approach: Models for noncovalent binding typically possess two major components: a method for generating possible bound conformations for the protein-ligand complex, and an energy function for predicting the stability of a given complex. A number of energy functions have been described in the literature. However, it is not clear how well the various ones work. A goal of the present project, therefore, is to develop and apply methods for testing the energy functions used in modeling protein-ligand interactions.

Results and Future Plans: A novel approach to testing energy functions has been developed. Given a protein-ligand complex whose conformation is known from experiment, and an artifically generated collection of plausible but incorrect conformations of the complex, an accurate energy function should assign the lowest energy to the correct conformation. An algorithm that generates plausible but incorrect conformations has therefore been developed. It has three basic steps: 1) randomization of the ligand conformation and orientation, independently of the protein; 2) placement of the ligand at an arbitrary spot in the known binding site; 3) optimization of the structure to a local energy-minimum. The algorithm has several novel features that will be described in a paper currently in preparation. The method is being used to test various models for long-ranged electrostatic interactions. One clear-cut result of this work is that much greater accuracy is achieved when all long-ranged interactions are included; i.e., when "cutoffs" are not used. In FY 1996, several simple energy functions were characterized in studies of three enzyme-inhibitor complexes of known structure. The CHARMM20 force field with a simple dielectric screening function is surprisingly effective at picking out the crystal structure in each case. The algorithm has been parallelized and test calculations have been carried out on a set of SGI workstations.

Our ultimate goal is not only to predict bound conformations, but also to predict binding affinities. A novel method for doing this has been developed. It involves computing the ratio of the configuration integrals for the free and bound ligand. The algorithm is described in a paper submitted to J.A.C.S. Preliminary tests show that convergence can be achieved for several interesting systems. Binding free energies computed with the energy function described in the previous paragraph are found to overestimate the experimental results about sixfold. We believe the chief source of error in these calculations is the use of an inadequate solvent model. Work in FY 1997 will focus upon optimization of the procedure and of the solvent model, and application to inhibitors of HIV protease. Time permitting, we will also pursue applications to more tractable host-guest systems.

Publications:

Antosiewicz, J., Gilson, M.K., McCammon, J.A., Wlodek, S.T., Clark, T.W., and Scott, L.W., "**Computer Modeling of Acetylcholinesterase and Acetylcholinesterase-ligand Complexes,**" Enzymes of the Cholinesterase Family, A.S. Balasubramanian, B.P. Doctor, P. Taylor, and D.M. Quinn, Eds., Plenum Publ. Corp., New York (1995).

Madura, J.D., Briggs, J.M., Wade, R.C., Davis, M.E., Luty, B.A., Ilin, A., Antosiewicz, J., Gilson, M.K., Bagheri, B., Scott, L.R., and McCammon, J.A., "**Electrostatics and Diffusion of Molecules in Solution: Simulations with the University of Houston Brownian Dynamics Program,**" Rev. Comput. Chem. (K.B. Lipkowitz and D.B. Boyd, Eds.) VCH Publishers, New York (1995).

Wlodek, S.T., Antosiewicz, J., McCammon, J.A, Straatsma, T.P., Gilson, M.K., Briggs, J.M., Humblet, C., and Sussman, J.L., "**Binding of Tacrine and 6-chlorotacrine by Acetylcholinesterase,**" Biopolymers 38, 109-117 (1996).

Marrone, T.J., Gilson, M.K., and McCammon, J.A. "**Comparison of Continuum and Explicit Models of Solvation: Potentials of Mean Force for Alanine Dipeptide,**" J. Phys. Chem. 100, 1439-1441 (1996).

12. Modeling Protonation Equilibria in Proteins

M.K. Gilson and J. Antosiewicz (University of Warsaw, Poland)

Objective: To develop predictive models for protonation equilibria in proteins, and to apply them to selected systems of biological interest.

Problem: The structure and function of proteins depend strongly upon pH, because pH determines the protonation states of ionizable groups. For example, the action of most enzymes depends strongly upon pH because ionizable groups in active sites must have defined protonation states in order to carry out catalysis. Theoretical studies of proteins therefore must include reliable methods for predicting protonation states.

Approach: The energetics of protonation in a protein are strongly modulated by the electrostatic environments of its ionizable groups. The Poisson-Boltzmann model for electrostatic interactions is being used to predict the charge states of ionizable groups in proteins. The accuracy of the models is tested by statistical comparison with measured pKas for a number of proteins. The more accurate models will be used in analyses of mechanism and inhibition of selected enzymes.

Results and Future Plans: Recent work has focused upon the effects of parameters, notably atomic charges and radii and the dielectric constant assigned to the interior of the protein. The atomic parameters used initially were not optimized for use with the Poisson-Boltzmann model. Recently, however, a new set of atomic parameters has been optimized for use with this model, based upon computation of the hydration energies of small molecules. It has now been demonstrated that these parameters yield more accurate pKas than the parameters originally used in the present studies. The possibility that the accuracy of pKa calculations might be increased by the use of solution structures of proteins derived from NMR studies, rather than crystal structures, has also been examined. Modest to substantial increases in accuracy are indeed observed, depending upon the choices of other parameters.

The most accurate model so far developed has been used to analyze the substantial measured pKa shifts of the acidic groups in turkey ovomucoid third domain. The picture that results differs significantly from that inferred by graphical examination of the structure. In brief, the calculations ascribe a much greater role to charge-charge interactions, and a much smaller role to hydrogen bonds with neutral polar groups. A paper in preparation will describe these studies.

In FY 1997, the present method was applied to several proteins G for which pKas are being measured in the laboratory of Dr. John Orban at CARB. The method has also been applied to additional groups in turkey ovomucoid third domain, with the plan of correlating with data from the laboratory of Dr. Andrew Robertson at U. of Iowa. Finally, a rather detailed study of the titration of the active site aspartyls of HIV protease in the presence and absence of inhibitors is underway. It is anticipated that these projects will be completed and written up in FY 1997.

Publications:

Antosiewicz, J., McCammon, J.A., and Gilson, M.K. **"The Determinants of pKas in Proteins,"** Biochemistry 35, 7819-7833 (1996).

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13. Determination of the Mechanism for the Activation of Transcription in E. Coli by cAMP Receptor Protein

F.P. Schwarz, I. Gorshkova (Russian Academy of Science, Russia), J.W. Brown, G.L. Gilliland, and S. Krueger

Objective: To determine how the binding reactions and structural changes in cAMP Receptor Protein (CRP) and its mutants preceding the activation of transcription in the cell control the level of transcriptional activity.

Problem: CRP is the key regulatory protein responsible for the expression of more than 20 genes that code the proteins metabolizing carbohydrates in *E. coli.* Upon binding to cyclic adenosine monophosphate (cAMP), CRP undergoes a conformational change. It binds specifically to the operon region of the DNA, inducing a bend. This complex interacts with RNA polymerase to generate the RNA sequence complementary to the DNA. Single amino acid substitutions at the cAMP binding site, about 20 Å away from the DNA and RNA Polymerase binding sites significantly affect the activation of transcription in the cell. It is not known as to how these mutations control the activation of transcription in the cell. This is important in understanding the interaction of DNA with proteins in general since the specific DNA binding motif of CRP is similar to that of other regulatory proteins, *e.g.* FNA activator protein, lambda cro repressor protein, and the cAMP dependent protein kinase in eukaryotes.

Approach: The level of activation of transcription by CRP will be compared to the levels of activation in the following mutated forms of CRP in the cell: the mutation of Thr127->Leu that results in activation by cyclic guanosine monophosphate (cGMP) and not cAMP; the mutation Ser128->Ala that results in the lack of transcriptional activity; and the double mutation, Thr127->Leu and Ser128->Ala, that results in transcriptional activation in the absence of the cyclic nucleoside ligands cAMP, cGMP, and cyclic inosine monophosphate (cIMP). Since it is unclear as to how these mutations alter the activity of CRP, the research described in this proposal will involve a two-pronged approach consisting of (1) identifying differences in the thermodynamic linkages between the binding events leading to transcription between the CRP/mutant complexes and (2) elucidating the nature of the conformational change by identifying structural differences between the cNMP-ligated CRP/mutants particularly in the operon and RNA polymerase-binding regions. The thermodynamic linkages will be determined from isothermal titration calorimetry and the structural differences from X-ray crystallography and small angle neutron scattering measurements.

Results and Future Plans: The level of transcriptional activation has been determined *in vitro* by monitoring the amount of a ribonucleotide sequence produced in a solution of the CRP/mutant, a cyclic nucleotide monophosphate derivative, ribonucleotides, and RNA polymerase. In this well defined assay, it was found that contrary to what is observed *in vivo*, the S128A and the T127L mutants activate transcription in the presence of cAMP. It was also observed that cIMP activates transcription in the double mutant as well as cAMP and that cGMP also activates transcription in the T127L mutant in the presence of cGMP as is observed in the cell. The thermodynamics of binding of the cycle nucleoside monophosphate ligands to CRP/mutants show, with the exception of cAMP binding to CRP, that the derivatives bind all with the same binding constants and enthalpies. The heat capacity changes accompaning this binding reaction, however, exhibit a rough correlation with the level of *in vitro* transcriptional activation, implying that large changes in the heat capacity are induced by a conformational change that is necessary for specific DNA binding. Small angle neutron measurements on CRP and the cAMP-CRP complex show that this conformational change results in a smaller, more compact complex. The thermodynamics of specific DNA binding were also determined from ITC measurements. The preliminary results show that the level of activation correlates very well with the binding affinity of the nucleoside-CRP/mutant complex, implying that this is the rate controlling step for activation of transcription. A preliminary structure of the cAMP-double mutant complex to 1.5 Å resolution from X-ray crystallography measurements show that the structure of the DNA binding region of this complex is very close to that of the cAMP-CRP complex, implying that specific DNA binding requires the same binding site motif in CRP and its mutants.

Research will continue in determining the binding thermodynamics of various specific DNA sequences and of RNA polymerase to the ligated CRP/mutant complexes to complete the determination of the thermodynamic linkages between the different binding events leading to the activation of transcription. This may or may not further substantiate that the concentration of ligated-CRP/mutant-DNA is the control step for the intiation of transcriptional activity in the cell. The structures of the various ligated mutant complexes will continue to be determined from X-ray crystallography to determine how well differences in the DNA binding region correlate with differences in the thermodynamics of DNA binding.

Publications:

Gorshkova, I., Moore, J.L., McKenney, K.H., and Schwarz, F.P., **"Thermodynamics of Cyclic Nucleotide Binding to the cAMP Receptor Protein and its Thr127→Leu Mutant,"** J. Biol. Chem. 270, 21679-21683 (1995).

Moore, J.L., Gorshkova, I., Brown, J.W., McKenney, K.H., and Schwarz, F.P., **"Effect of cAMP Binding Site Mutations on the Interaction of cAMP Receptor Protein with Cyclic Nucleoside Monophosphate Ligands and DNA,"** J. Biol. Chem. 271, 21273-21278 (1996).

14. *Expression of G-Protein Coupled Receptors in* Pichia Pastoris *Yeast Cells*

K.D. Ridge, N.G. Abdulaev (Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia), M.P. Popp (University of Florida), and W.C. Smith (University of Florida)

Objective: To functionally overproduce opsins and structurally related G-protein coupled receptors in a yeast expression system.

Problem: The overproduction of opsin and structurally related membrane bound receptors in a variety of heterologous expression systems has so far proven quite difficult and consequently limited both biophysical and structural investigations. While production of microgram quantities of opsin for biochemical studies in various mammalian cell systems has been achieved, expression in other heterologous systems capable of producing larger amounts of functional protein have met with limited success. The goal of this research is to determine whether the methylotrophic yeast *Pichia pastoris* is capable of producing opsin and other G-protein coupled receptors in the quantities required for the above-mentioned studies.

Approach: The strategy we have adopted to express bovine opsin in *Pichia pastoris* yeast cells relies on the fusion of an amino-terminal secretion signal sequence to opsin in order to target the protein to the plasma membrane of the yeast cell. This is advantageous since previous studies have shown that only plasma-membrane-inserted opsin is capable of binding 11-*cis*-retinal to form the rhodopsin chromophore. Additionally, endogenous yeast proteases cleave the secretion signal sequence from the opsin once it is appropriately inserted in the membrane. This allows one to express the opsin devoid of any additional amino acids.

Results and Future Plans: The present results show that stable yeast transformants express bovine opsin to a cell mass of ~0.3 milligrams per liter. The opsin is located exclusively in the membrane and displays a molecular mass and glycosylation pattern that is characteristic of the protein isolated from natural sources. Further, the membrane bound opsin forms the rhodopsin chromophore with 11-*cis*-retinal and shows spectral and functional charactersitics indistinguishable from that of the native photoreceptor. Thus, *Pichia pastoris* yeast cells have the capacity to express G-protein coupled receptors in quantities sufficient for biophysical and structural studies. Further efforts to improve the yield of chromophore formation and to develop a large scale purification protocol are currently under investigation.

Publications:

Abdulaev, N.G., Popp, M.P., Smith, W.C., and Ridge, K.D., **"Functional Expression of Bovine Opsin in the Methylotropic Yeast Pichia Pastoris,"** Protein Express. Purif. (in press).

15. Folding and Assembly in Visual Rhodopsin

__K.D. Ridge__, S.S.J. Lee (University of Maryland), and N.G. Abdulaev (Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia)

Objective: To identify regions of the opsin polypeptide chain that contain sufficient information to fold independently, insert into a membrane, and assemble to form a functional photoreceptor.

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 folding and assembly of the photoreceptor are far from being understood at the molecular level. The long term goal of this research is to understand the process(es) by which rhodopsin, and receptors of related function, fold into a ligand-binding and signal-transducing component structure.

Approach: The strategy we have adopted to provide a detailed analysis of the folding and assembly of rhodopsin focuses on the identification and characterization of independent protein-folding domains. It is well known from studies on a number of soluble globular proteins that certain polypeptide fragments behave as independent folding domains and that many of these fragments can be functionally recombined. In this study, opsin fragments have been generated by genetic manipulation of the bovine opsin gene and examined for their ability to fold and assemble into a retinal-binding protein after coexpression in mammalian cells.

Results and Future Plans: The present results show that the coexpression of two or three complementary fragments allows the formation of a noncovalently bound rhodopsin. These findings strongly suggest that the functional assembly of rhodopsin is mediated by the association of multiple protein-folding domains. Additionally, while many of the opsin polypeptide fragments contain sufficient information to fold and insert into a membrane, only those separated at specific locations along the polypeptide chain assemble to form a functional photoreceptor.

Publications:

Ridge, K.D., Lee, S.S.J., and Abdulaev, N.G., **"Examining Rhodopsin Folding and Assembly Through Expression of Polypeptide Fragments,"** J. Biol. Chem. 271, 7860-7867 (1996).

16. *The Biological Macromolecule Crystallization Database: Development of Crystallization Strategies*

J.E. Ladner, M. Tung, and G.L. Gilliland

Objective: To use the NIST/NASA/CARB Biological Macromolecule Crystallization Database (BMCD) to develop strategies for facilitating the search for the conditions for growing suitable crystals of a biological macromolecule for X-ray diffraction studies.

Problem: The search for crystal growth conditions of a biological macromolecule traditionally requires trying a variety of reagents over a wide range of concentrations, temperature, and pH. Depending on the number of reagents used and how finely the parameter ranges are varied, the number of experiments needed to find suitable conditions may require months to carry out. Several systematic procedures and strategy suggestions have been put forth, but no *universal* strategy for searching for the crystal growth conditions for a biological macromolecule has gained widespread acceptance.

Approach: The availability of the crystallization data for all classes of biological macromolecules in the BMCD make a unique resource for developing crystallization strategies. Four different categories of crystallization problems have been identified: 1) the crystallization of a previously crystallized macromolecule, 2) crystallization of a variant macromolecule or ligand-macromolecule complex related to a macromolecule that has previously been crystallized, 3) crystallization of a macromolecule that is homologous to a previously crystallized one, and 4) crystallization of a unique macromolecule unrelated (in the polymer sequence) to anything that has been previously crystallized. The data in the BMCD is used in the development of strategies for each of these classes of problems to facilitate the production of crystals for diffraction experiments.

Results and Future Plans: Algorithms employing the use of the BMCD for each of the problems mentioned above have been developed. In each case the BMCD is used to select the parameters and the parameter values, or range of values, which will be used in the initial screen for each case. The utility of the methods for specific cases for each of the four different problems have been tested in the crystal growth laboratory of CARB. Crystals have been obtained in each case indicating the utility of the procedures. Further testing, optimization, and automation of the procedures are planned before incorporating these into the World Wide Web version of the BMCD.

Publications:

Gilliland, G.L. and Ladner, J.E., ***"Crystallization of Biological Macromolecules for X-ray Diffraction Studies,"*** Current Opinion in Structural Biology 6, 595-603 (1996).

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Gilliland, G.L., Tung, M., and Ladner, J.E., ***"Development of Crystallization Strategies Using the Biological Macromolecule Crystallization Database,"*** Proceedings for the IUCr Workshop on Crystallization (in press).

17. *Hybrid Bilayer Membranes*

A.L. Plant, C. Meuse, V. Silin (Institute of Biochemistry, Lithuanina Academy of Sciences, Lithuanaina) and D. Vanderah

Objective: To investigate a novel supported lipid bilayer, which has a structure analogous to the membranes of living cells, for its application as a rugged, biomimetic matrix for active membrane proteins and for the study of cell-surface reactions.

Problem: The characteristics of lipid membranes such as their insulating properties, self-assembling components, dynamic responses, and the fact that they provide a matrix for membrane proteins, make them interesting for their potential applications in biocatalysis, sensors, electronic, and electrooptic materials. Commercial uses of lipid membranes in industrial applications have been limited by difficulties associated with their fabrication and stability and by the difficulties associated with understanding membrane protein structure and function.

Approach: We have developed a hybrid bilayer membrane (HBM) system consisting of both natural (phospholipids) and synthetic (alkanethiol) components, which is easily formed by self assembly, is supported on a conductive metal surface, and is stable for very long periods of time. This cell-membrane-like material is rugged and easily fabricated, which allows practical applicability to industrial-scale use. One focus of this project is to use this membrane mimetic system as a research tool for studying the structure and activity of membrane proteins. This system may provide one of the few ways to achieve high resolution structural information on membrane proteins which in general, are intractable to crystallography. In addition, it provides an important experimental tool for reconstituting and studying the function of transmembrane proteins such as cell surface receptors, light harvesting proteins, transport proteins, and redox enzyme complexes.

Results and Future Plans: This stable and rugged membrane can be formed and studied in aqueous conditions and in dry air. A wide number of measurement techniques can be applied which provide highly quantitative information on the system, as well as a comparison of hydration and dehydration states on the structure of the bilayer. In-depth structural analysis of this model membrane by vibrational spectroscopy is providing a complete picture of the similarity of these membranes to other models of cell membranes. Comparison of surface-enhanced Raman and Fourier transform infrared spectroscopy results has provided details on the subtle changes which occur in the alkanethiol layer when a bilayer is formed. Reflectance-absorbance IR spectroscopy is providing high quality data on the structural details of the phospholipid components. These studies augment work performed in collaboration with MSEL on neutron reflectivity measurements of HBMs which is providing some of the first data on single bilayer membranes, including high resolution data on the location of a pore forming toxin, melittin, in the bilayer. Our CRADA partner, SmithKline Beecham, is using HBMs for monitoring the activity of thrombosis-effecting agents at the cell membrane surface by surface plasmon resonance. In-house surface plasmon resonance measurements are being employed to understand the mechanism of spontaneous self assembly of HBMs from phospholipid vesicles. In the coming months, the incorporation of transmembrane proteins into HBMs will become a focus of this project.

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18. Bacteriorhodopsin Research

H. Weetall, B. Robertson, and A. Druzhko (Institute of Theoretical and Experimental Biophysics, USSR Academy of Sciences, Russia)

Objective: To develop methods for the study of bacteriorhodopsin (bR), a bacterial transmembrane protein that functions to convert light energy into metabolic energy. bR is of industrial interest for its potential use in molecular electronics applications, and fully successful use of this material will require a better understanding of how to modify its spectral and kinetic properties.

Problem: The photocyle of bR begins with the absorption of light by the associated chromophore and ends after the release of a proton and return to its original ground state. Developments that are essential to the use of bR as a viable electrooptical material include learning how to control the kinetics of the photocycle, and increasing the range of spectral sensitivity. Better understanding of the relationship between bR structure and function may eventually permit modifications of the protein to result in more favorable optical or temporal characteristics.

Approach: Two approaches have been taken to increase our knowledge of the mechanism of bR action. The first approach is modification of the protein sequence through genetic mutations. We have obtained mutants of bR that contain substitutions in the amino acids directly involved in proton uptake and release. We are presently examining these mutant bR molecules for their spectral characteristics and proton pumping activity. The second approach is to substitute analogs for the native retinal chromophore, in order to alter the wavelengths at which the protein absorbs light. These protein, containing chromophore analogs, show changes in kinetics, spectral response, and proton release and uptake.

Results and Future Plans: To quantify the effect of the changes, we have developed a method that allows direct measurement of the proton-pumping activity of bR. The techique employs a tin-oxide electrode that responds directly to pH changes. This method is sufficiently sensitive to detect the light-induced response of a single layer of membrane patches containing bR, and on Langmuir-Blodgett films of oriented bR. It is the first tool of its kind that allows direct measurement of the function of bR or other membrane proteins because it responds only to uptake and/or release of protons, and not to charge separation. With this tool, we have been able to quantify the effect of sequence and/or chromophore changes on proton uptake and release by the protein. Results indicate that chromophore substitution has a much more dramatic effect on proton pumping than does sequence alteration. We are presently examining the characteristics of several new bR analogs including fluorinated and de-methylated derivatives. These data will help us develop a model for the mode of action of the chromophore. If successful, we will be able to synthesize chromophores that will provide bR with spectral and kinetic characteristics, making them more useful for industrial molecular electronics applications.

Publications:

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19. Self-Assembling Nanoscale Pore-Forming Proteins: Potential Applications in Separations and Sensing

J.J. Kasianowicz

Objective: To understand an important class of naturally occurring pore-forming proteins, which will aid in producing rationally designed components for the separation and sensing of a wide variety of water-soluble analytes.

Problem: Nanoscale self-assembling pore-forming proteins function by opening passages for certain ions and macromolecules in otherwise impermeable membranes. These structures, or biomimetic structures like them, could play a role in separation and sensing applications. We are currently developing pores to detect, characterize, and separate two types of molecules: polymers (e.g., DNA) and heavy metal divalent cations. Improving the ease and efficiency of selecting, separating, and identifying the sequence of DNA segments is of interest for both forensic and diagnostic applications. Better ways of detecting and removing heavy metal ions will benefit many manufacturing applications.

Approach: A combination of electrophysiological techniques and genetic engineering (in collaboration with H. Bayley's group at the Worcester Foundation for Biomedical Research) is being used to redesign and adapt proteins that self-assemble to form well-defined nanoscale pores in lipid bilayers. The ability of polymers or cations to interact with these pores is determined by the changes in the pore's ionic current caused by these analytes.

Results and Future Plans: Our recent work on the interaction of DNA and RNA, nonelectrolyte polymers and cations with the ion channel formed by *Staphylococcus aureus* α-hemolysin (α-HL) suggest that nanoscale pores could be used in separation and sensing applications. First, in collaboration with D.W. Deamer (UC Santa Cruz) and D. Branton and E. Brandin (Harvard University), it was demonstrated that single stranded (but not double stranded) RNA and DNA polynucleotides could be driven through an ion channel by an applied electric field. The passage of individual polynucleotides through the pore caused brief blockages in the ionic current that otherwise flowed freely. The duration of the current blockages was proportional to the length of the polynucleotides. Secondly, in collaboration with S. Bezrukov (NIH), it was shown that an ion channel can discriminate between differently-sized poly(ethlyene glycol) nonelectrolyte polymers and govern the time the polymer is associated with the pore. Thirdly, in collaboration with H. Bayley's group, a combination of genetic engineering and electrophysiological techniques were used to redesign and adapt α-HL pores that exhibit different sensitivities to different heavy metal divalent cations.

The crystal structure of the α-HL pore was recently determined by others to 0.19 nm resolution. This will greatly aid the design of pores with the desired properties for separation and sensing applications. In addition, we are interested in producing pores with different diameters for characterizing and separating different sized polymers.

Publications:

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20. Dynamic Process Modeling of Biomolecular Materials

J.B. Hubbard

Objective: To develop theoretical constructs to improve our understanding of dynamic biomolecular processes. This enables us to predict reaction pathways and kinetic parameters for association of biomolecules, and aids us in the design and interpretation of experiments. Interaction dynamics between biological molecules in solution and at surfaces are controlling features of sensor operation, diagnostic tests, cellular recognition events, cellular motion, and the formation of modified surfaces such as organized biomolecular materials.

Problem: Macromolecular interactions are complex by virtue of the many internal degrees of freedom of the molecules themselves, and the resulting number and complexity of inter- and intramolecular spatial and temporal correlations. To be able to predict complex biomolecular behavior, we must devise ways of identifying and evaluating the dominant underlying thermodynamic and kinetic components.

Approach: The complexity of these problems frequently precludes the use of conventional methods that work well for simpler physical systems. Conceptual economy drives the choice of modeling approach and involves consideration of the physical complexity of the system and the limitations of detail in the experimental methods. Appropriate approaches tend to involve probabilistic models. Models are based on continuum mechanics, statistical thermodynamics of equilibrium and metastable systems, and occasionally lead to algorithm design and computer simulations. Much of this effort involves close collaborations with experimentalists.

Results and Future Plans: Three kinds of modeling problems have been recently addressed. 1) Through the use of hydrodynamics and Brownian dynamics, we devised an accurate and efficient method of correlating the solvent-accessible shape of a macromolecule with its experimentally-determined diffusion coefficient. In collaboration with Dr. Jack Douglas (MSEL), Dr. Jim Given (CARB), and Dr. H. Zhou (University of Hong Kong), a simple relationship between the hydrodynamic friction of an arbitrarily-shaped Brownian particle and its electrostatic capacitance has been developed. An algorithm has been designed which accurately calculates this capacitance for objects (such as model macromolecules) of shapes ranging from small clusters of intersecting spheres to bead-string models of long-chain polymeric molecules.
2) A probabilistic theory based on a system size expansion of a master equation was developed which gave generic formulas for the surface retention times for multivalent binding in the case of liposomes binding to a modified surface. This approach, which led to the deduction of the precise nature of the chemical binding cooperativity in this experimental model system, continues to be refined for predicting cooperative binding and detachment mechanisms for other multivalent molecular recognition scenarios.
3) Observations of large scale cooperativity in biological membranes provided the basis for a generalized model for the effect of intermolecular interactions on triggering phase transitions in proteins immobilized at surfaces, and in some instances leading to their freezing in metastable phases of highly correlated molecular conformations. The implications of this model to the denaturation of non-specifically adsorbed proteins at surfaces continues to be explored.

Publications:

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21. DNA and Enzyme Sensor Applications

S. Glazier and J. Horvath

Objective: To develop strategies and methods for the successful application of DNA and enzyme-based sensors. Significant technical challenges are associated with the application of biological molecules in devices. In the two projects described below, fundamental basic science studies are brought closer to application such as environmental sensing.

Problem: Macromolecules including DNA and enzymes are examined in various configurations as potential components of electrochemical and optical devices. Engineering of prototype devices allows us to consider real world issues such as fabrication and adaptation of instrumentation and methodology to field applications. Environmental monitoring is a particularly important application for biosensors. Two classes of analytes, organic pesticides and nitrate ions, have been targeted.

Approach: Fluorescence polarization was used to quantify aromatic pollutants that compete with fluorescent dyes for DNA binding sites. This work was carried out with partial support from the U.S. Environmental Protection Agency.

Through a CRADA with Nitrate Elimination Co., strategies are being studied for successful application of enzyme-based electrodes that respond to the presence of nitrates.

Results and Future Plans: The sensitivity of fluorescence polarization in this DNA-containing assay system was sufficient to warrant the testing of a large number of EPA priority compounds by this method. All EPA-targeted pesticides were found to intercalate into calf-thymus DNA with similar levels of sensitivity. In addition, an evanescent wave device, suitable to field measurements, was modified to allow detection of pesticides by monitoring fluorescence intensity. These results have been transferred to the EPA for further development of these techniques.

For nitrate ion detection, a highly stable, water soluble nitrate reductase from corn seedlings was examined as a possible component of a sensing scheme. Electrodes were constructed which respond to nitrate ion concentration at levels near or below the 10 mg/L limit set by the EPA for nitrate in drinking water. Eventual application for this work may be in the design of sensors for testing the safety of drinking water. Electrodes with self-contained electron-transfer mediators were developed for this application. A series of alkylviologen compounds were synthesized, and while it was determined that they are not appropriate for application to the water-soluble nitrate reductase, they will be important for faciliating electron transfer of membrane-bound enzymes. In the next phase of this work, a membrane-bound nitrate reductase electrode will be constructed.

II. 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 devices, instrumentation, and mathematical models required for analysis, control, and optimization of industrial processes. The Division's research seeks fundamental understanding of, and generates critical 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.

Programs:
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, national 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, leak rate, liquid density and volume, and air speed measurements,** with more than 1000 standard tests and calibrations performed this year. The Division's commitment to provision of these services involves many facets: the establishment, maintenance, and improvement of the primary standards; continuing intercomparisons of these standards to those of other nations; development of suitable mechanisms that allow customers in the field and in secondary calibration laboratories to realize requisite measurement accuracy; and continual attention to service efficiency and measurement quality.

While these 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**. In FY 1996, these efforts included development of improved thermocouples for control of thermal processing equipment including: rapid thermal processing (RTP) systems; low-range gas flow standards and calibrations, evaluation and performance modeling of residual gas analyzers used to monitor gaseous contamination in fabrication tools; and development of methods to determine plasma electrical properties (joint with the very low-level moisture concentration Electronics and Electrical Engineering Laboratory and the Physics Laboratory) and measurements of contamination control in process gases. In these efforts we typically make use of a reference chemical processing reactor prototypical of industrial manufacturing environments. This allows critical tests of the measurement approach and its utility for the intended application. Because processing systems are typically complex, with strongly coupled chemistry and mass transport characteristics, 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 all elements identified by the Semiconductor Industry Association's (SIA) National Technology Roadmap for Semiconductors. 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 NIST's NSMP, seeks to develop **benchmark chemical mechanisms and supporting thermochemical and kinetic data, for equipment and process design**. These chemical mechanisms must be integrated with appropriate transport models and simulations, and validated to meet the needs of our industrial customers. Again, reference reactors serve as vital testbeds for validating these models.

The technical approach described above emphasizes development of measurement technology, provision of process models and supporting data validation on prototypical systems, characterizes not only our semiconductor-related efforts, but also is representative of our process technology related research in the areas of materials chemistry, spray combustion, and hydrothermal oxidation. The first of these, highly synergistic with the semiconductor models and data program, develops **chemical and process models and data to support the production of high technology materials.** In a project joint with the Materials Science and Engineering Laboratory, we are studying multiphase combustion (oxidation) synthesis of nanoscale magnetic oxides, important for information storage technology. Another focus area for the program is the use of a sodium-burner to synthesize metals and non-oxide ceramics, e.g., SiC, required for high-temperature, high-strength applications in the aerospace industry. This novel processing method offers a relatively low temperature (low energy consumption) route with promise for bulk-synthesis of these generally difficult-to-prepare materials. It also has the advantage of essentially zero emissions providing an inherently "green" processing approach for the production of high technology materials.

Addressing industrial problems associated with the operation and design of thermal reactors is the main focus of the other two Division programs in process technology. Based upon a unique combination of spray measurement capabilities and a laboratory-scale prototype spray combustion facility, the first 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 primary objective of this effort is to **establish the validity of computational models for burner performance and to establish a means to correlate performance with operating conditions** (with a strong emphasis on the atomization process). The industrial use of hydrothermal oxidation (HTO) as a zero-emission treatment technology for aqueous wastes requires further development in the areas of processing conditions and unit operations. Division **research in HTO technology** addresses the measurement and data issues associated with salt formation and deposition, metallic corrosion, and heat transfer in near-critical fluids. Development of *in-situ* (optical) detection methods for monitoring corrosive species (reactants and products) at the temperatures and pressures characteristic of HTO is also very important to the electric power industry. Babcock and Wilcox, a major process engineering firm, is collaborating with us, under a Cooperative Research and Development Agreement (CRADA), to research a solution to this measurement problem.

A significant part of the Division's work is in the area of chemical sensor technology with two research efforts: **micromachined gas sensor arrays** and **sensing applications of self-assembled monolayers (SAMs).** The first effort is collaborative with the Semiconductor Electronics Division of the Electronics and Electrical Engineering Laboratory. The technology is based on CMOS-fabricated SiO_2 bridge structures formed with silicon micro-machining to make 'micro-hotplate' arrays. Chemical sensing capability is achieved by depositing metal oxides, e.g., SnO_2, and surface-dispersed catalytic metal-additives to form robust, electrical-conductance based sensing elements on the micro-hotplates. Because of their small mass the micro-hotplates can be heated to near 1000 °C and cooled back to ambient temperature with time constants in the millisecond range. This enables temperature programmed sensing (TPS), which exploits adsorption/desorption kinetics, to enhance the sensitivity, selectivity, and repeatability of the sensing response. Combined with selection of metal oxides and catalysts, TPS arrays offer excellent potential for real-time sensing of multi-component gas mixtures. NIST holds two patents on the fabrication and application of these devices. To date, more than 26 companies have contacted us to discuss licensing this technology. 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 which could limit commercial application.

The self-assembly of alkanethiol monolayers, of the general formula $X(CH_2)_nSH$, on the surfaces of noble metal substrates is the focus of the second major element of the Division's sensor program. These molecules attach via the thiolate headgroup to form strongly chemisorbed and densely packed assemblies with surfaces having terminal-group (i.e., the moiety 'X') controlled functionality. The SAMs can be two-dimensionally patterned, for example via a NIST patented photo-process, to produce arrays of two or more alkanethiol molecules with different terminal functionalities selected to enhance or prevent physical or chemical-adsorption of specific biological or organic molecules. Our research is currently focused on the attachment of active DNA probes with the goal to develop the means to reliably produce the desired SAM arrays, including studies of the roles of chemical and structural "forces" in the self-assembly process, and to develop approaches to sensing and identifying molecular recognition events, such as DNA hybridization. SAM based sensors hold great promise for applications in medicine, pharmacology, and biotechnology.

Selected Program Highlights:
The Division has responsibility to provide and disseminate the International Temperature Scale of 1990 (ITS-90) over the range 0.65-1235 K. A major goal of NIST's **temperature standards program** was to realize ITS-90, as defined, over this complete range. We have now reached this goal with completion of the portion of the scale which relies on gas thermometry in the range 3-24.6 K. NIST's achievements in this area place us in the lead among all national standards laboratories in both the range of, and low level of uncertainty in, our realization. We also have a competence project, joint with the Physical and Chemical Properties Division and the Optical Technology Division of the Physics Laboratory, to reduce (by factors of 3 to 8) the uncertainty of **thermodynamic temperature measurements** in the range above 500 K. The plan is to extend high accuracy acoustic thermometry from nominally 303 K to 933 K (the Al-point). The Optical Technology Division will link to our measurements at the Zn-(693 K) and Al-points and then extend them to the Au-point (1337 K) with expanded uncertainty approaching 10 mK. In practical process control applications, thermocouples (TCs) are the most widely employed of all thermometers. This year we completed an investigation leading to the development of **Pt/Pd TCs** as very accurate and stable TCs for high temperature applications. They have demonstrated remarkable (±20 mK) reproducibility for temperatures up to 1200 K. These devices will be increasingly important in semiconductor manufacturing which requires very accurate temperature control in wafer processing furnaces. In a new effort this year, we are developing **thin film thermocouple thermometers** to provide accurate surface-temperature measurement for control of RTP systems used in semiconductor manufacturing. The Pt/Pd couple has evolved as the initial material of choice for this application.

The Division provides the national standards and calibration services for **pressure and vacuum measurements** over 16 decades of pressure, from 10^{-7} to 10^9 Pa. Recent advances in the measurement of pressure and vacuum include the development of new standards and calibration services as well as significant improvements in instrumentation. The Division is engaged in a collaboration with DH Instruments, Inc. to characterize the **world's first 50 mm diameter piston gauges**; these large-diameter gauges have the potential to become primary standards in the important range near atmospheric pressure. A new service for calibrating gas-operated piston gauges up to 110 MPa also was initiated this year.

In the general area of fluid transfer measurements, the Division provides **gas flow and leak rate standards** and calibrations over the range from 10^{-14} to 10^2 mol/s and similar services for liquid flows in the range from 10^{-3} to 10^3 l/s. To support measurement needs in industries such as semiconductor manufacturing, **new high accuracy primary standards for gas flows in the range 10^{-7} to 10^{-3}** mol/s are under development. A constant volume device, having relative expanded uncertainties at or below 0.1% (coverage factor of 2), recently has been completed and a constant pressure device is planned for completion in FY 1997. A secondary, transfer standard, based on a unique high pressure- differential laminar flow element, also has been developed and shown to have relative expanded uncertainties of approximately 0.1% when used as a transfer standard.

We completed this year construction of a major new **calibration and testing facility for gas flowmeters for vehicle exhausts**. This facility, developed in response to the flow measurement needs expressed to NIST by the American Industry/Government Emissions Research (AIGER) consortium, provides gas flow conditions simulating those of automotive exhausts. The combination of high moisture content and high temperatures (to 700 K) are unique characteristics of this flow measurement facility. Calibrations are required in the flow range of 1 to 36 standard (10^5pa at 273.15 K) liters per second (0.04 $\text{mol}\cdot\text{s}^{-1}$ to 1.6 $\text{mol}\cdot\text{s}^{-1}$). We have also completed a **new liquid volume calibration facility** under joint sponsorship by the American Petroleum Institute. The new facility achieved or exceeded it's design goals to provide a four-fold reduction in calibration uncertainty. Work under an **air speed proficiency testing program**, with instrument manufacturers participating under a CRADA, has progressed to the point that testing in the participants air speed calibration facilities will commence in early FY 1997. Finally, research continues under two CRADAs, involving the Electric Power Research Institute and meter manufacturers, to investigate the use of **non-intrusive acoustic flow meter technology** for improved accuracy in measurements of cooling-water flows in power plants.

A major new thrust in the Division is to develop **species specific, partial-pressure measurement methods and standards** to calibrate instrumentation used to monitor process gases. This effort will expand vacuum metrology beyond the traditional inert gases to include identification and quantification of low-level contaminants in higher-pressure background gases. Such contaminants play a critical role in determining the quality of vacuum-processed components and represent a major challenge from the point of view of real-time process monitoring and control. Our initial focus is on the optical measurement technique **cavity-ring-down-spectroscopy** which holds promise for low concentration measurements of moisture in vacuum, perhaps down to the 10^{-8} Pa range, and for measurements of trace concentration of water in gases, e.g., humidity. Work this year resulted in the development of a first principles description, based on stable resonator theory, for the frequency and time-domain response of cavity-ring-down signals. This work filled an important gap in the understanding of this very powerful (and promising) technique. The theoretical analysis has provided a much needed basis for quantitative description of the cavity-ring-down signals. The motivation for this work, and the low gas flow rate standards discussed above, comes in part from the Division's commitment to support the measurement needs of the semiconductor industry. Other efforts in this area include proficiency tests of the calibration systems operated by manufacturers of mass flow controllers and the study of the metrological **characteristics of residual gas analyzers (RGAs)**, widely used to monitor gas purity in semiconductor fabs. This application of RGAs is severely hampered by the extreme non-linearities in sensitivity found in some instruments; for example, changes by two orders of magnitude in ion current, at fixed partial pressure of the contaminant, as the overall system pressure is changed. A model developed to explain our data for these instruments suggests methods to minimize these effects in commercial RGAs and critical design criteria to minimize the non-linearities in future RGAs. In many areas of our measurement support for the semiconductor industry, we are now transferring technology to the industry. Hardware and software that we have developed in recent years to make accurate measurements of RF electrical parameters in the **Gaseous Electronics Conference (GEC) cell** are now finding application in industry. We also have provided experimental validation of a plasma sheath model which allows prediction of ion flux and kinetic energy at the wafer surface (critical parameters determining etching and damage rates) from measurement of external RF voltage and current waveforms. Our recently developed **chemical kinetic mechanism (i.e., thermochemical data for a set of species and rate constants for a set of chemical reactions) for high temperature fluorocarbon chemistry**, which has application in the areas of plasma etching and polymer formation, has recently been made available on the World Wide Web. This **interactive database**, entitled **CKMech**, will greatly enhance the availability and utility of the data, and significantly reduce the time demands on our staff for answering questions about this widely-used new information. We have made significant progress this year in the development of a **model for micro-contamination in a spinning-disk thermal CVD reactor**. This reactor, currently being installed in our laboratory, will serve as the reference reactor for study of thermal CVD processing widely employed by the semiconductor industry. We recently started a CVD modeling effort that focuses on realistic chemical kinetics in addition to (the more typical) energy and mass transport. A 60-step reaction mechanism which incorporates both silane decomposition and particle nucleation has been developed. The conditions of growth relevant to micro-contamination are very different from those typically encountered, for example, in multiphase routes to the growth of nanophase materials. One consequence is that species condensation on particles has been found to be a dominant mode of particle growth. This has a number of possible consequences, some highly beneficial such as reduced particle impaction on the wafer, which need to be validated in the experimental program.

This year we published a thorough study of the **properties of silicon nanoparticles** (clusters up to 1000 Si atoms) derived from molecular dynamics simulations. The size and temperature dependence of the cluster energy, internal structure, diffusion coefficients, phonon density, and related properties have been revealed for the first time. Research in the materials chemistry area also had yielded results of potential importance to semiconductor processing. A unique Na-vapor based process for essentially complete destruction of CFCs (the end products are halogen salts and carbon) has been developed and patented. This chemistry has promise for treating the effluent of plasma-processing reactors.

The major portion of the modification and construction effort in the spray combustion program was completed in FY 1996. This work involved construction of an enclosure for the spray burner to allow emission measurements, and installing and testing an array of on-line chemical and soot measurement systems which will run in parallel with the spray characterization facilities. Measurements to characterize the performance of an **acoustically driven atomizer** developed by Fluid Jet Associates (a CRADA partner) and a **steam-assisted atomizer** for use with variable composition waste fuels, also were tested as part of this program. In the **HTO program**, we made two key findings with regard to the relative **performance of hydrogen peroxide (H_2O_2) versus oxygen in hydrothermal oxidation systems**. First, we have resolved a longstanding inconsistency in the HTO literature between the similarity and dissimilarity of these two oxidants. We established that H_2O_2 is not highly labile. For H_2O to behave similarly to oxygen, it must first be fully decomposed (T> 400 °C, > 3 s residence time, in contact with stainless steel). At milder conditions, H_2O_2 decomposition is incomplete causing its behavior to deviate from that of pure oxgen. This led to our second finding, that H_2O_2 is the superior oxidant. This was established by reactions with ammonia and acetic acid, important refractory products from many waste streams. From below 720 K to over 970 K, the peroxide activation energy was nearly 260% smaller than with oxygen, which results in substantial performance advantages. For example, at 720 K, the rate constant for acetic acid conversion with peroxide is nearly 90 times greater than that with oxygen. For the same reaction rate that with oxygen, the system must be run 130 K hotter. This imposes additional energy and material requirements upon HTO systems. In a continuing collaboration with MIT, we used a special optical cell to study the process of **salt formation in the vicinity of a hot wall in near-critical water**. It was found that (Na_2SO_4) salts deposited only by heterogeneous nucleation at the hot surface with no precipitation in the bulk (even in the presence of added Al_2O_3 particles to serve as nucleation sites). This result supports the MIT model which suggests that supersaturation does not occur because heat and mass transfer in salt solutions near the critical point are much more gas-like than previously assumed. Expressed in terms of the Lewis number (ratio of thermal to mass diffusivities) , a change of between 2 and 3 orders of magnitude relative to ambient salt solutions in water is needed to explain these results.

A very important development in the **micromachined gas-sensor arrays** program this year was the successful replacement of aluminum with a tungsten-titanium alloy in the metal layers which form the heat distributor plate and the electrical contacts to the sensing film. The tungsten replacement is more chemically robust, allows a higher temperature range to better exploit adsorption/desorption kinetics, and (most importantly) provides a much more reliable electrical contact to the sensing film. These tungsten-based devices have significantly enhanced our research and testing projects. Another important development this year is an approach to optimization of the temperature sequence employed in temperature programmed sensing. This rather general optimization method (subject of a patent disclosure) provides a prescriptive route to differentiation of the characteristic response pattern of chemically similar species. The approach was demonstrated to provide very distinct responses for ethanol and methanol which previously had been difficult to distinguish. We also have extended self-registered thermal CVD, based on temperature control of individual micro-hot plates, to produce multi-element array sensors with different sensing films and metal overlayers (catalysts) on each element of the chip. These devices greatly aid in the development and optimization of methods for detection and quantitation of multi component mixtures.

As part of our sensing applications of self-assembled monolayers (SAMs) program, we have developed (and applied for a patent) **a new process for immobilizing single stranded DNA (ss-DNA) probes on Au surfaces**. Based on previous successes with self-assembly of thiol derivatized ss-DNA, this new method allows precise control of the concentration of ss-DNA in a two-component SAM. This surface immobilized ss-DNA is highly specific, i.e. hybridizing only with complementary DNA, and, when the coverage is optimized, the layers have shown an exceptional 50% hybridization efficiency, a factor 2-5 larger than previously reported. This enhancement is attributed to achieving an optimal packing density which reduces steric hindrances to binding. In addition, the method eliminates non-specifically bound ss-DNA and the surface-immobilized ss-DNA shows reversible hybridization, which can be undone via melting, and is still active and specific following the melting treatment. These measurements of layer coverage and hybridization have required application and adaptation of a wide range of analysis techniques (x-ray photoelectron spectroscopy, IR and Raman spectroscopy, secondary ion mass spectrometry, scanning tunneling microscopy (STM) and others) to the characterization of these surface immobilzed bio-molecular systems. In related work, we published the results of a study of the process of self-assembly of alkane thiol monolayers on Au by use of direct STM imaging. Molecular images of the growing layers, formed via gas phase dosing, showed that the molecules first form a two-dimensional lattice gas, then transition to low-density island clusters, and finally undergo a surface phase transition involving reorientation of the alkyl chains to form the dense mono-molecular film most familiarly known as SAM.

Awards in FY 1996:

Dr. Charles R. Tilford was elected a Fellow of the American Vacuum Society "For outstanding leadership and technical contributions in the development of world-class programs on vacuum, low pressure, and low gas flow rate measurements and standards." This prestigious election to Fellow status reflects the quality and impact of Dr. Tilford's pioneering work in vacuum science and technology over the years. Mr. Stephen Doty and Mr. John Houser received the NIST Measurement Service Award this year. Mr. Doty's award was for the development of the new high-pressure gas-operated piston gauge calibration service, while Mr. Houser was recognized for his efforts in developing the Division's new volume calibration facility. Dr. Gregory Poirier was awarded the CSTL 1996 Technical Achievement Award for his pioneering studies of the process of self-assembly of alkane thiols on Au surfaces. Dr. Frank DiMeo was recognized with the NIST Sigma Xi Award for the Outstanding Poster in the Annual Sigma Xi Postdoctoral Symposium, 1996.

Organizational Structure:

In FY 1996 the Division was expanded to include the Pressure and Vacuum Group (from the former Thermophysics Division). We now have six Groups: Fluid Flow, High Temperature Processes, Reacting Flows, Process Sensing, Thermometry, and Pressure and Vacuum. The following pages present more detailed discussions of some of the accomplishments and plans for the coming year in each of the Division's programs.

B. Selected Technical Reports

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

G.J. Rosasco, V. Bean, B.W. Mangum, G.E. Mattingly, S.A. Tison and J.R. Whetstone

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

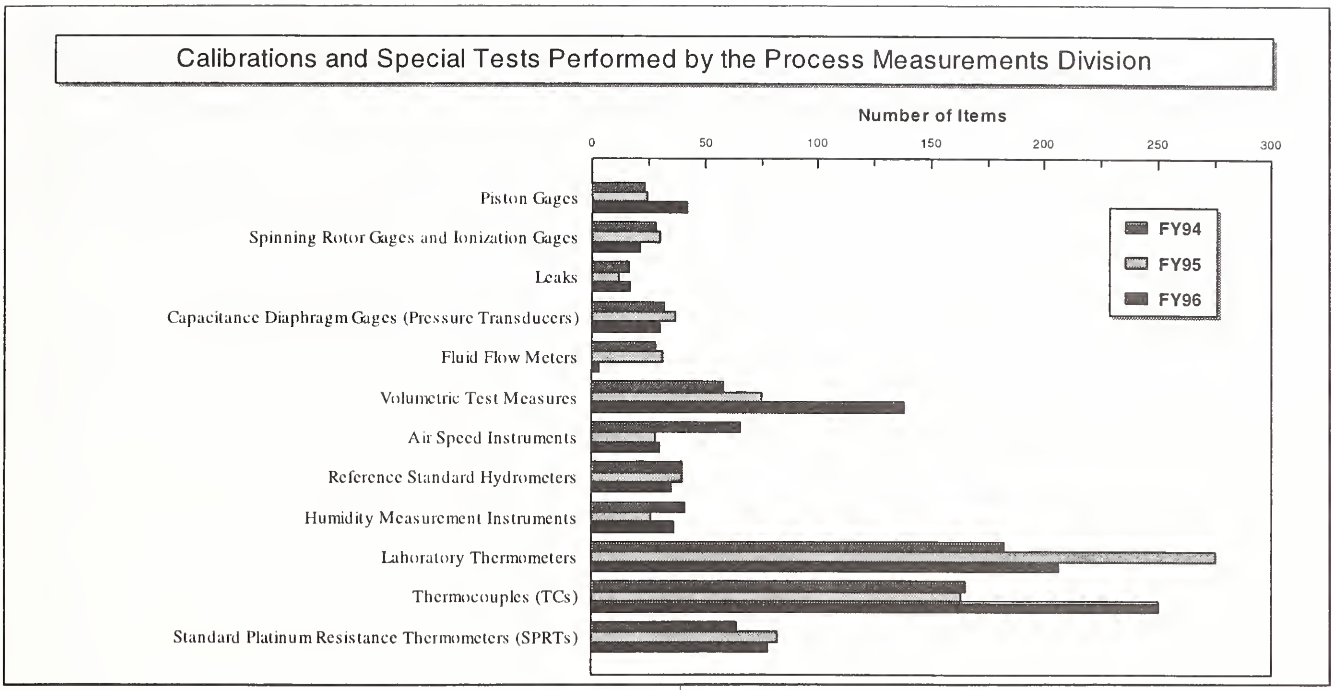
Problem: The Process Measurements Division is responsible for realizing, maintaining, and disseminating the national standards for measurement of temperature (in the range 0.65-1235 K), pressure, vacuum, leak rate, humidity, fluid flow rate, liquid volume and density, and air speed. Issues of primary importance in this area involve assessing and meeting, to the extent practicable, customer requirements for accuracy in their measurements, 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: Our capabilities, experimental techniques and the facilities used to provide these services are being upgraded, with concomitant improvements in efficiency and in expanded uncertainties. Activities in the thermometry area during FY 1996 included the completion of the automation of the calibrations of liquid-in-glass thermometers, industrial platinum resistance thermometers, thermistor thermometers, and of thermocouples in the Liquid-in-Glass Calibration Laboratory. Additionally, a small comparison system for the calibration of "used" high-temperature standard platinum resistance thermometers (HTSPRTs) that probably have been contaminated by the customer during use was constructed, evaluated, and put into service. This system will be used to compare customer HTSPRTs against a NIST reference HTSPRT over the temperature range 550 °C to 1075 °C, or at approximately the freezing points of Al and Ag.

In the pressure and vacuum area, we have constructed and calibrated a low-pressure transducer package which will be used in an international intercomparison of pressure standards sponsored by the Consultative Committee on Mass of the CIPM. We also instituted a new, high-pressure gas-operated piston gauge calibration service that extends the calibration range from 16 MPa to 280 Mpa. The amount of staff time dedicated to routine calibration workload has been reduced through increased automation of the calibration services and increased efficiency in the reporting of the results. An automated flow-generation system for the orifice-flow standards used in the calibration of ionization gauges was constructed this year. Final testing, calibration, and implementation of this system is planned for FY 1997. Other plans include the development of differential pressure measurement capability in the range of 1 pa to 1 kPa, the incorporation of a new gas piston gage pressure standard, and expansion of pressure calibrations from the current 280 MPa to 500 MPa.

In the area of humidity measurements, construction of a new gravimetric hygrometer, part of the effort to re-establish and extend our primary moisture measurement capability, was completed. This instrument, based on state-of-the-art refinements of traditional gravimetric humidity measurement approaches, will enable 0.1% relative expanded uncertainty measurements in the range from +40 °C to approximately −42 °C frost point temperature. This system also will serve as a reference for our newest instrumental developments which seek to establish a primary standard for humidity measurements based on optical absorption. Construction of a new low-frost-point humidity generator, designed to provide very low


Calibrations and Special Tests Performed by the Process Measurements Division

moisture concentrations, with frost points to −100 °C (mole fraction of 10^{-8}), also was completed. Ultra-low-moisture-content standards are required to calibrate measurement instrumentation used to assure contaminant-free process gases in the semiconductor industry. This system uses all metal seals to assure very high gas purity and employs heat-pump and thermoelectric cooling technology, thereby eliminating potentially harmful and expensive refrigerants. Temperature stability of approximately 5 mK in the range from -100 °C to -20 °C has been demonstrated, far better than design specifications. Both these new systems will be tested and uncertainty levels established in FY 1997. Other activities in the humidity area include an eleven nation international intercomparison, which NIST is chairing under the auspices of the Consultative Committee on Thermometry of the CIPM. Participants come from Europe, North America, and Asia. This year we completed characterization of an optical hygrometer for the Asian Pacific region which will allow comparison of realizations of humidity standards from −70 °C to +40 °C dew point temperatures. We also completed a second intercomparison of humidity generators in the 1-10 mg/kg range with participants from the semiconductor industry under the auspices of Semiconductor Instruments and Materials International (SEMI).

New efforts in the area of standards for fluid transfer, involving volume standards and a new simulated vehicle-exhaust gas flow calibration facility, are discussed in the reports following. Other efforts this year involve the upgrading of our hydrocarbon flow calibration facility to achieve relative expanded uncertainty of 0.025%. This calibration service, which provides support vitally important to US jet engine manufacturers for their testing activities, and to DoD engine performance testing facilities, is based on a passive-piston volumetric device originally purchased by the U.S. Navy. This year's effort focused on making all subsystems operational, upgrading electronics and software components, and replacing all malfunctioning flow control hardware. The system was returned by the contractor who was performing the repairs and upgrades and is expected to be fully operational in FY 1997. In the area of flow measurement proficiency testing, we have conducted tests in several industrial laboratories to quantify the traceability of their water flow meter calibration capabilities to our standards. These tests were done using transfer standards consisting of tandem turbine flow meters; several sizes of meters were used to span desired water flow rate ranges from 200 L/min. to 16,000 L/min.

In the area of air speed measurements, a new proficiency testing program involving round robin testing with meter manufacturers and secondary calibration laboratories has been initiated as part of an Air Speed Proficiency Testing CRADA. This program grew out of requests from industry to resolve measurement discrepancies existing among different calibration facilities. Workshops with industry at NIST established the need to assess the performance of air speed calibration facilities through round robin testing and produced the selection of transfer standards and the desired test conditions which span the range from 0.3 m/s to 15 m/s. Twelve laboratories, representing instrument manufacturers, DOD, the electrical power industry, and the chemical process industry, are participating. The goal of this program is to establish traceability to the NIST primary standard in all the participating laboratories. It is expected that this effort will provide the basis for a new international intercomparison of air speed measurement standards.

Publications:

Abbott, P.J. and Tison, S.A., "**Commercial helium leak standards: Their properties and reliability**," J. Vac. Sci. Technol. A 14(3), 1242 (1996).

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Filippelli, A.R., "**Influence of Envelope Geometry on the Sensitivity of "Nude" Ionization Gauges**," J. Vac. Sci. Technol. A 14, 2953 (1996).

Terao, Y., Takamoto, M., and Mattingly, G.E., "**Preliminary Intercomparison of Anemometer Calibration Systems at Very Low Speeds between the National Standards Laboratories in Japan and the USA**," Japanese Society of Mechanical Engineers, International Journal (in press).

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

B.W. Mangum, C.W. Meyer, W.L. Tew, D.C. Ripple, G.F. Strouse, G.W. Burns, K.M. Garrity, J.D. Melvin, C.D. Vaughn, G.T. Furukawa (Guest Researcher), D. Licea-Panduro (CENAM, National Center for Metrology, Querétaro, Mexico), E.Y. Xu (Guest Researcher), and K.S. Gam (KRISS, Korea Research Institute of Standards and Science, Taejon, Korea)

Objective: To realize the International Temperature Scale of 1990 (ITS-90) as it is defined over the range from 0.65 K to 1235 K, to investigate the non-uniqueness of the ITS-90 over this temperature range, to compare our realization of the ITS-90 with those of the national standards laboratories of other countries, and to disseminate the scale to the user community.

Problem: The ITS-90 extends upward from 0.65 K. The realization of this scale, as it is defined, has been completed at NIST over the range from 83.8058 K to 1234.93 K, as reported in "Guidelines for Realizing the International Temperature Scale of 1990 (ITS-90)," NIST Technical Note 1265, U.S. Govt. Printing Office, Washington, DC 20402, August 1990, and in several papers published in: *Temperature: Its Measurement and Control in Science and Industry*, Vol. 6 (Edited by J. F. Schooley), New York, American Institute of Physics, 1992. The realization of the ITS-90 over the range from 0.65 K to 83.8 K, however, has not been completed at NIST. Consequently, NIST still uses a "wire scale", based on some old NBS work and on some thermometers calibrated by NPL, as the NIST reference for calibrations in the region 0.65 K to 83.8 K. This wire scale consists of a standard Platinum Resistance Thermometer (SPRT)-based scale spanning the region 13.8 K to 83.8 K and used primarily for calibration of SPRTs, and a rhodium iron resistance thermometer (RIRT) based scale spanning the region 0.65 K to 27.1 K and used primarily for calibration of RIRTs and germanium resistance thermometers. Recent measurements at NIST indicated that the wire scale may be in error by about 1 mK in certain regions of the scale below 25 K. Realization of the ITS-90 below 83.8 K will free us from this wire scale and this will reduce our uncertainty of calibrations in that range. There are other uncertainties resulting from the non-uniqueness of the scale over the range from 1.25 K to 1234.93 K, however, that have not yet been evaluated. The magnitudes of these non-uniquenesses must be determined in order to arrive at the total uncertainties of realization and dissemination of the scale. Also, new and/or better methods for dissemination of the ITS-90 (apart from calibration of thermometers) are needed to get the scale to users requiring high accuracy.

Approach: The completion of the implementation of the ITS-90 will be accomplished through the realizations of the temperature of the triple-points of Ar (83.8058 K), O_2 (54.3584 K), Ne (24.5561 K) and equilibrium hydrogen (e-H_2) (13.8033 K) in large non-transportable cells and in transportable cells suitable for use in international intercomparisons; measurements of the saturated vapor pressures of ^{3}He (0.65 K to 3.2 K), ^{4}He (1.25 K to 5.0 K) and H_2 at (17.035 ± 0.010) K and at (20.270 ± 0.010) K; and interpolating-constant-volume gas thermometry (ICVGT) from 3.0 K to 24.5561 K, using ^{3}He or ^{4}He gas as the working fluid. To achieve an expanded uncertainty in temperature of $\leq$0.1 mK, the pressure must be measured with an expanded uncertainty of $<30 \times 10^{-6}$ (for vapor pressures) and with an Type A standard uncertainty of $< 3 \times 10^{-6}$ (for gas thermometry). The gas pressures are measured with a gas-lubricated piston gauge in series with a differential capacitance diaphragm gauge. The non-uniqueness of the ITS-90 will be determined by investigating its different sources in the various temperature ranges and determining its magnitude over those different ranges. In some cases, e.g., for the range from 933 K to 1235 K, a large number of thermometers are required for this investigation. Consequently, collaborations with national laboratories of other countries are desirable, or necessary, in order to have enough thermometers for a meaningful investigation. The ITS-90 is disseminated through the calibration of thermometers, SRM fixed-point cells of pure materials, SRMs of pure metals of the defining fixed-point materials, and SRM thermometers.

Results and Future Plans: Our goal of the realization of the ITS-90 from 0.65 K to 24.5561 K was reached this year. This realization was at high precision and the scale in that range was retained on two SPRTs and/or three RIRTs, depending on the temperature range. The realizations by vapor pressures of ^{3}He and ^{4}He showed a non-uniqueness, i.e., defect, approaching 0.3 mK in parts of the region of overlap of those definitions of the ITS-90, i.e., between 1.25 K and 3.2 K. Additionally, the non-uniqueness in the range of overlapping helium vapor-pressure and ICVGT from 3.0 K to 5.0 K was determined to have a maximum value of about 0.6 mK. Temperatures determined by ICVGT and by H_2 vapor pressures at 17.035 K and at 20.270 K differed by 0.7 mK and -0.4 mK, respectively. The temperatures from ^{3}He, ^{4}He, and e-H_2 vapor-pressure realizations differed from temperatures on our wire scale by as much as 0.8 mK; outside the expected agreement. The non-uniqueness of the scale in the range from 13.8033 K to 24.5561 K arising from the use of SPRTs and ICVGT will be completed in FY 1997. Also, additional thermometers will be calibrated for use in international comparisons.

For use in realization, and for international intercomparisons, of the ITS-90 through the use of sealed transportable cells of the cryogenic gases; triple-point cells of Ne were constructed; baked under high vacuum at high temperatures; filled with high-purity gas from a high-pressure; high-purity gas-handling system; and sealed by a pinch-weld technique developed for these cells. Results of preliminary measurements on the cells indicated that the new design of the cells led to significant improvements in the behavior of sealed cells. Additional triple-point cells of e-H_2 and Ne, and if time permits, O_2 and Ar, will be constructed and evaluated during FY 1997. The cells will be used in calibration of thermometers by the fixed-point method and also used in international intercomparisons to ascertain the agreement of our realization of the ITS-90 fixed points with those of other national standards laboratories. Such intercomparisons are the basis for bilateral agreements between the USA and other countries on the equivalence of our respective realizations of the scale.

In FY 1997, the Thermometry Group will coordinate, under the aegis of the CCT, an international intercomparison of national realizations of the ITS-90 among those countries that are members of the CCT and that have the competence and ability to realize the scale at the highest level of accuracy in the range from 83.8058 K to 933.473 K. In addition, we will participate in the other CCT-organized international intercomparisons of the ITS-90 in its other ranges of temperature below 1234.93 K, i.e., from 0.65 K to 24.5561 K, from 13.8033 K to 273.16 K, and from 933.473 K to 1234.93 K. These are two-year projects.

Efforts to determine the non-uniqueness of the ITS-90 in the ranges 933.473 K to 1234.93 K and 273.16 K to 933.473 K were continued this year. These data are required in order to establish realistic uncertainty statements for calibrations. Since a large sample of high temperature standard platinum resistance thermometers (HTSPRTs) is required for this study and since our supply of HTSPRTs is inadequate for the range 933 K to 1235 K, we organized a collaborative effort with the national laboratories of Italy, Instituto de Metrologia "G. Colennetti", and the Netherlands, Van Sinden Laboratory. Seven of the HTSPRTs of NIST 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, underwent comparison measurements between 873 K and 1094 K in our high-temperature comparator, for which alterations and evaluations for optimal performance were made. ***Preliminary*** measurements for the 7 HTSPRTs indicate a maximum value of non-uniqueness (approximately 2 mK) that is significantly smaller than that observed at IMGC for their set of HTSPRTs. The investigation was interrupted because the heaters in our comparator developed open circuits. These heaters are being replaced and work on this project will continue next year, with completion of the investigation of the NIST HTSPRTs and the beginning of work on the IMGC HTSPRTs by the end of FY 1997.

In recent years, we have had a significant effort in developing fixed-point cells and in certifying high-purity metals for use in preparing fixed-point cells, with the goal of disseminating the ITS-90 or providing materials for use in its realization to secondary calibration laboratories. In this effort, certification of 99.999 99% pure Indium for SRM 1745 (Indium freezing-point standard for use in constructing freezing-point cells used in calibrating SPRTs) and for SRM 2232 (Indium differential scanning calorimetry melting-point standard for use with differential scanning calorimeters was completed. These SRMs will soon be available for purchase. In FY 1997, high-purity Sn will be characterized as SRM 741a for use in preparing freezing-point cells for realizing the ITS-90.

Another method of disseminating the ITS-90 to secondary calibration laboratories and others requiring high accuracy is through a new SRM of calibrated capsule SPRTs, covering the range from 13.8033 K to 429.7485 K, that will be developed in FY 1997. The dissemination of the ITS-90 to those requiring high accuracy at high temperatures may be through highly-stable and highly-accurate thermocouples (TCs) that require infrequent or no recalibration. In this regard, 20 Au/Pt TCs that will be made available as SRM 1749 and that ***will not require recalibration*** were constructed and their certification begun. This SRM will be completed in FY 1997.

Publications:

Meyer, C.W. and Reilly, M.L., "**Realization of the ITS-90 at the NIST in the Range 0.65 K to 5.0 K using ^{3}He and ^{4}He Vapour-pressure Thermometry**," Metrologia 33, 383-389 (1996).

Mangum, B.W., Pfeiffer, E.R., Strouse, G.F., Valencia-Rodriguez, J., Lin, J.H., Yeh, T.I, Marcarino, P., Dematteis, R., Liu, Y., Zhao, Q., Ince, A.T., Cakiroglu, F., Nubbemeyer, H.G., and Jung, H.J., "**Intercomparisons of Some NIST Fixed-Point Cells with Similar Cells of Some Other Standards Laboratories**," Metrologia 33, 215-225 (1996).

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Mangum, B.W., Furukawa, G. T., Meyer, C. W., Reilly, M.L., Strouse, G. F., and Tew, W.L., "**Realization of the ITS-90 at the National Institute of Standards and Technology**," Proceedings of TEMPMEKO '96, 6th International Symposium on Temperature and Thermal Measurements in Industry and Science (in press).

Meyer, C.W. and Reilly, M.L., "**Realization of the ITS-90 at NIST in the Range 3.0 K to 24.5561 K Using an Interpolating Constant Volume Gas Thermometer**," Proceedings of TEMPMEKO '96, 6th International Symposium on Temperature and Thermal Measurements in Industry and Science (in press).

Tew, W.L., "**Sealed Cells for the Realization of Triple Points at the National Institute of Standards and Technology**," Proceedings of TEMPMEKO '96, 6th International Symposium on Temperature and Thermal Measurements in Industry and Science (in press).

3. *High-Temperature Thermocouple Research and Sensor Development*

G.W. Burns, D.C. Ripple, K.G. Kreider, B.W. Mangum, D.P. DeWitt (Purdue Univ.) and M. Battuello (IMGC)

Objective: To develop new sensors, e.g., wire and/or thin-film thermocouples, as reference thermometers for secondary calibration laboratories and/or as fairlyinexpensive, high-accuracy, high-stability, high-temperature thermometers for industrial use, including use in surface-temperature measurements.

Problem: Two problems being addressed: 1) the lack of stable thermocouples (TCs) to serve as reference standards in secondary calibration laboratories and/or as high-quality thermometers for industrial applications. TCs currently used as reference standards (types S, R and B, all of which contain Rh), and as the best-available thermometers for most industrial applications, have, at best, at the time of calibration, total expanded uncertainties of about 0.1 K at the Au freezing point (1337 K). These increase rapidly with increase in temperature above 1337 K to about 1.6 K at 1725 K. Also, due to unstable inhomogeneities and selective oxidation of Rh, the emfs of these TCs drift with use, perhaps an increase of as much as a factor of 10 at 1337 K. 2) unreliable measurements of surface temperatures caused by use of traditional contact thermometers or non-contact radiation thermometers, e.g., in the semiconductor industry. Accurate, high-speed measurements of temperatures of surfaces are especially critical in semiconductor wafer preparation by rapid thermal processing (RTP) because accurate control of temperature during short, high-temperature exposures is critical to product quality and device performance.

Approach: To address the need for stable TCs to serve as reference standards in secondary calibration laboratories and/or as high-quality thermometers for industrial applications, TCs of pure noble metals, such as Au, Pt, and Pd, are being investigated. These metals can be produced at exceptionally high purity (5-6 nines) and are generally resistant to oxidation in high temperature environments. Since the TCs are constructed of these pure metals, inhomogeneities are small, defects can be essentially removed by annealing, and the TCs are stable and interchangeability is excellent. To address the need for accurate measurements of temperatures of surfaces, the feasibility of thin-film TCs is being investigated. These devices become part and parcel of the surface and thereby obviate the uncertainties associated with conventional contact thermometers (uncertainty of the quantitative relationship of the measured temperature and the surface temperature) and with radiation thermometers (uncertainty with respect to the time-dependent, effective emissivity of the surface).

Results and Future Plans: In our efforts to develop stable, high-accuracy TCs, Au/Pt TCs have been found to be an excellent choice for temperatures up to 1275 K. We have developed a reference function for them and we are producing the Au/Pt thermocouples as SRM 1749. The TCs for that SRM were constructed this year and their certification will be completed in FY 1997. In an effort to develop a TC for use to about 1775 K, the stability of Pt/Pd TCs as a function of annealing procedures has been investigated up to 1675 K; some have been heat treated at 1375 K for more than 1000 h. Variations of 5 mK to 10 mK per 100 h at 1375 K were obtained. Also, inhomogeneities equivalent to only 4 mK to 16 mK were observed. In a collaborative project with IMGC to determine the reference function for these TCs, experiments were conducted on six Pt/Pd TCs to obtain emf-t_{90} data up to about 1775 K. Those TCs had been constructed and evaluated at NIST for that purpose. NIST performed the measurements from 273 K to 1337 K (by fixed points using the ice point and the freezing points of In, Sn, Zn, Al, Ag, and Au; by comparison against an SPRT from the ice point to 723 K; and by comparison against three calibrated Au/Pt TCs from 693 K to 1273 K). IMGC performed measurements from 1073 K to 1775 K (by comparison against a radiation thermometer). The reference function is necessary in order to facilitate the use of these TCs in industrial applications and as high-temperature transfer standards. The analyses of the data of these TCs up to 1775 K will be completed, and the reference function developed, in FY 1997.

Investigation of techniques for preparation of stable thin-film TC arrays for accurate temperature measurements, with high spatial resolution (a few micrometers) and short response times (of the order of a millisecond), continued this year. Also, an apparatus suitable for calibration and stability testing of thin-film TCs from 273 K to 1275 K was put into service. In the project for developing improved surface temperature measurements in the semiconductor manufacturing industry (especially for RTP), some of the most promising metals with regards to their feasibility as stable thin-film thermocouple materials at elevated temperatures on silicon wafers were evaluated. This involved evaluating the behavior of their thin films at various temperatures up to 1100 K, as this is a critical part of the selection process for the development of procedures for fabrication of thin-film thermocouples on silicon wafers. From tests on Pt, Pd, and Ir thin films, Pt and Pd were chosen as the first candidates, at least for the initial stages for temperatures up to about 1100 K. Sputter deposition and annealing procedures were developed to optimize performance to 1100 K. In collaboration with the Optical Temperature and Source Group of the Optical Technology Division, instrumentation is being designed for the first test wafer (that will be obtained commercially). We will deposit thin-film Pt/Pd TCs on wafers then it will be partially instrumented with small-wire TCs. This instrumented wafer will be our first trial in determining temperatures of Si wafers more accurately than is currently realized in industrial practice. For this wafer, several different types of sensors (different TCs and a radiation thermometer) will be used for determination of the temperatures. For this project, a new laboratory is being set up by the Optical Technology Division and measuring equipment required for the project has been ordered. In order to quantify the uncertainty in the calibrations of thin-film thermocouples in the calibration apparatus, the heat flow in the test coupons containing the thin-film thermocouples will be modeled. In addition to this thin-film work, Pt/Pd TCs of the conventional types discussed above and suitable for applications to about 1775 K in diffusion furnaces used by the semiconductor industry have been developed. There are plans to have industrial trials of these Pt/Pd TCs next year.

Publications:

Burns, G.W. and Ripple, D.C., "**Techniques for Fabricating and Annealing Pt/Pd Thermocouples for Accurate Measurements in the Range 0 °C to 1300 °C**," Proceedings of TEMPMEKO '96, 6th International Symposium on Temperature and Thermal Measurements in Industry and Science (in press).

4. *Thermodynamic Temperature Measurements*

D.C. Ripple*, B.W. Mangum, R.A. Moldover (Syracuse Univ.), K. Gillis (838) and M. Moldover (838)*

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

Problem: There remain unresolved inconsistencies in previous measurements of thermodynamic temperatures at 500 K and above by two groups of researchers at NIST (each group used a different gas-thermometry apparatus). Those inconsistencies led to significant 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: Thermodynamic temperatures between 500 K and 933 K will be measured by acoustic techniques, using spherical resonators. Acoustic thermometry relies on the relationship of the speed of sound in an ideal gas to the thermodynamic temperature. Speed of sound measurement via spherical resonator techniques, previously applied to the high-accuracy determination of the Boltzmann constant, will be extended to high-temperature operation. Gas purity will be controlled to a very high degree, a major challenge in this high-temperature environment. 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 5 to an total expanded uncertainty of 10 mK. The results will have expanded application to radiometric measurements of temperature to much higher temperatures (upwards 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.

Results and Future Plans: This project to improve the accuracy of thermodynamic temperature measurements above 500 K, using acoustic resonators between 500 K and 933 K, was started in FY95. During FY 1996, it was decided that the stainless steel resonator used for determination of the gas constant would be used at temperatures up to about 775 K. The gas-handling and temperature-control systems suitable for operation at 775 K were constructed. The temperature of the resonator will be controlled to 1 mK. The temperature fluctuations due to purging of the resonator will be at the tenths of millikelvin level. Also completed was the design, construction and extensive characterization of acoustic transducers (source and detector) suitable for high-temperature use; capacitance diaphragm type transducers (using very thin discs of pure Si) were selected. The transducers were tested in Ar gas at pressures from 25 kPa to 1 MPa in a specially-designed coupler and pressure vessel. Additionally, engineering calculations and design of the pressure vessel (and associated feedthroughs) which will enclose the resonator were made, as was a thermal analysis of a furnace with low thermal gradients. Although the calculations on the pressure vessel were made specifically for stainless steel for temperatures up to 775 K, they may be easily extended to other materials and for higher temperatures. The furnace on which the thermal analysis was made, and which will be used in the measurements with the stainless steel resonator noted above, is for use up to 775 K. A furnace of a different design will be required for use at higher temperatures. The electronics necessary for acoustic generation and detection were assembled in FY 1996. Tests of oxidation and creep of possible resonator materials at temperatures up to 775 K were conducted also, with the result that for stainless steel, creep will not be a problem and the oxidation can be controlled.

In FY 1997, an acoustic resonator suitable for operation at temperatures as high as 775 K will be assembled, the high-stability furnace for the resonator will be constructed, the entire thermodynamic temperature measurement system assembled, and preliminary measurements near room temperature will be obtained.

5. *Comparison of International Pressure Standards*

S. Tison, *A. Miiller, J. Schmidt, and C. Tilford*

Objective: To establish the level of agreement between national pressure and vacuum standards for pressures between 3×10^{-7} Pa and 500 MPa.

Problem: International trade depends on the equivalence of measurements. One of the largest segments of industrial measurements are pressure and vacuum. To alleviate technical trade barriers the relative agreement of national measurements standards needs to be determined and maintained.

Approach: A May 1996 meeting of the Consultative Committee for Mass and Related Quantities (CCM) identified six 'key' international comparisons in pressure and vacuum;

1) 3×10^{-7} to 1×10^{-3} Pa (absolute)
2) 1 Pa to 1000 Pa (absolute)
3) 1 Pa to 1000 Pa (differential)
4) 5 kPa to 120 kPa (absolute)
5) 50 kPa to 7000 kPa (gauge)
6) 50 MPa to 1000 MPa (gauge)

The NIST Pressure and Vacuum Group will participate in all of these comparisons, is serving as pilot laboratory for two of the comparisons, and during the past year was actively involved in three of the comparisons.

Results and Future Plans: Last year's report presented initial results from Key Comparison 1. These showed excellent agreement between NIST and the Physikalische-Technische Bundesanstalt - Berlin (PTB), but, to within limits imposed by unstable transfer standards, indicated significant differences with the National Physical Laboratory - Teddington (NPL). Repeat measurements during 1996 confirmed the difference with NPL, but were also plagued by transfer standard instabilities. At a meeting of the Low-Pressure Working Group of the CCM (chaired by C. Tilford) it was agreed that better transfer standards must be found before proceeding further. NIST agreed to test and provide new transfer standards, and act as pilot laboratory for the next round of comparisons. Equipment has been procured and assembled for the new transfer standards. Initial NIST calibration of the new transfer standards is scheduled to begin in early 1997.

NIST is also acting a pilot laboratory for Key Comparison 2, but testing this past year has found large instabilities in the Capacitance Diaphragm Gage (CDG) transfer standards. During the past year we have also tested three new low-range commercial transducers, two of which are microelectronic-machined silicon-diaphragm devices. Initial testing has found their stability to be as much as two orders of magnitude better than that of CDGs, but the commercial units are not designed for absolute pressure measurements, and they lack the sensitivity to be useful at the very lowest pressures. However, we believe they can be combined with the CDGs to give transfer standard performance that is significantly improved over that obtainable with the CDGs alone. We are negotiating with suppliers to obtain units that can be modified for absolute pressure use. We hope to assemble and test a new transfer standard package before the end of 1997.

NIST has completed and submitted its comparison measurements for the first phase of Key Comparison 5 (extending up to 1000 kPa), but will not have results until other participants complete their measurements.

6. *Quantitative Optical Measurements of Gas Partial Pressures*

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

Objective: To develop measurement standards for partial-pressure measurements of low-level gaseous contaminants, with particular emphasis on H_2O, CO_2, CO, O_2, H_2, and CH_4

Problem: Low-level gaseous contaminants cause deleterious effects in many chemical processing systems. There is increasing demand for improved measurement instrumentation and primary standards at low concentration. As an example, The Semiconductor Industry Association National Technology Roadmap identifies water vapor concentration measurements at the nanogram per kilogram level as one of the key metrological parameters for contamination-free manufacturing in the next devices generation. Such a measurement capability is required by the year 2001. Traditional techniques for generating low partial pressures of active gases, including water, are severely limited due to surface interactions and long time constants. Furthermore, the technical approaches used in the development of existing standards for vacuum and humidity are not capable of meeting many of the identified industrial measurement requirements. The development of a fundamentally new approach to this measurement problem is required.

Approach: Develop quantitative high sensitivity optical absorption measurements, coupled with the expertise and unique measurement capabilities of the Pressure and Vacuum and Process Sensing Groups to establish a new primary standard for low-levels of gaseous contaminants. Cavity-Ring-Down Spectroscopy (CRDS) has been identified as the most suitable approach to acheive quantitative low-level gas density measurements. The centerpiece of CRDS is a high-Q Fabry-Perot etalon. By measuring the Q of the cavity, one obtains directly the optical losses within the cavity. The introduction of an absorbing species into the cavity degrades the cavity-Q, and the change in Q is a direct measure of the gas absorption. The cavity Q is measured by exciting the cavity with pulsed laser radiation, monitoring the subsequent photon decay, and measuring the decay time constant. The decay time constant is a direct measure of the cavity Q. The realization of this approach has been enabled by recent advances in high quality mirrors used to form the optical cavity. Using these ultra low-loss mirrors, cavities with Q's as high as 10^{10} have been constructed. These are expected to provide ultimate water partial pressure detection limits below 10^{-6} Pa.

Results and Future Plans: During the past year, we addressed several issues critical to the development of a quantitative measurement capability using CRDS. The primary issue here was the development of a predictive model for the time and frequency response of ring-down cavities to pulsed laser excitation. The models presented heretofore were not predictive; they did not account for the mode structure of the ring-down cavities, nor did they incorporate the structure of the excitation laser field and its influence on ring-down signals. We applied the well-established theory of optical resonators to yield a predictive model for the electromagnetic fields in ring-down cavities, the radiant flux of light exiting the cavity (the signal), and the cavity transmittance as a function of laser pulse structure and detuning. The details of the cavity construction (mirror reflectance, mirror size, and separation) were encompassed in this model. Concomitant with this effort, a number of experiments were conducted explicitly to test certain predictions of our analysis. The resulting model can be used to guide the design of optical cavities, to help in the selection of laser light sources, and to optimize of experimental configurations. Furthermore, this theory has been incorporated into our data analysis and will be used in the development of a substantiative uncertainty analysis. These results of this work have been published.

Other experiments conducted during the past year were intended to demonstrate quantitative measurements of gas densities using CRDS. From these measurements, we have achieved a detection limit of $< 3 \times 10^{-8}$ cm^{-1} (absorptivity), which corresponds to a water vapor concentration equivalent to a vapor pressure of 10^{-5} Pa in vacuum. Further improvement in the implementation of this technique are projected to yield a 10 to 100 fold improvement.

Publications:

Hodges, J.T., Looney, J.P., and van Zee, R.D., "**Response of a Ring-Down Cavity to an Arbitrary Excitation**," J. of Chemical Physics 105, 10278-10288 (1996).

7. *Liquid Volume Measurement Standards*

V.E. Bean and J.F. Houser

Objective: To provide improved calibration services for volume test measures up to 100 U.S. gallons within total expanded uncertainty of 0.01%.

Problem: To meet its goals of improved measurements in the field, the American Petroleum Institute (API) has requested NIST to reduce its relative expanded uncertainty for the calibration of volume test measures by a factor of four, from 0.04% down to 0.01%. Volume test measures are used to calibrate instrumentation for determining the flow rate in oil pipelines. As the U.S. uses 1 billion cubic meters of oil per year (18 million barrels per day) at a value of $110 billion, the factor of 4 improvement in the NIST Standard can correspond to significant amounts of oil and of dollars in custody transfers.

Approach: NIST has constructed a new primary standard facility for liquid volume measurements. Volumes are determined by weighing the distilled water required to fill a test measure up to the reference mark, measuring the water temperature, and calculating the volume from the density equation for water. The technique determines contained volumes and establishes limits for errors due to transfer. This new facility is a cost-shared project with API.

Results and Future Plans: The new facility became fully operational in FY 1996. The expanded uncertainty in the calibration of a 100 gallon test measure in this new facility is 0.0031%, exceeding the goal of 0.01% by a factor of three and improving on the previous expanded uncertainty by a factor of twelve. The expanded uncertainties of a given design of test measure are larger for the smaller test measures. The uncertainties in the calibration of 5 gallon test measures in this new facility range between 0.004% and 0.017% depending upon the design.

With continued support from the API, we plan to extend the range of the facility to 500 gallons and to measure thermal expansion coefficients of test measures.

8. *Fluid Mechanics and Flow Metering Research*

G.E. Mattingly, T.T. Yeh, P.I. Espina, J.C. Mu, J.D. Wright, and J.M. Allingham

Objective: To investigate and expand the capabilities of computational fluid dynamics (CFD) and ultrasonic flow measurement technology and assess their potential for improving flow measurements in "non-ideal" meter installation conditions which can lead to significant flow metering errors.

Problem: Flow meters can be very inaccurate when the velocity profile entering the meter is not that for which the meter is designed. Meters are designed to measure the "fully-developed" equilibrated distribution of flow in a pipe that is produced by "ideal" conditions of long, straight lengths of constant diameter pipe preceding the meter. However, because of space restrictions and process piping requirements, industrial meter installation designs cannot usually arrange "ideal" conditions. Furthermore, when flow meters are retrofitted into existing piping systems to improve measurements, installation conditions are invariably very different from ideal. These situations can produce significant errors in material accountability. To remedy this, *in situ* calibrations or calibrations in duplicate piping are currently the only alternatives, but these are seldom feasible. This is particularly true in the electric power generation industry where large diameter, high flow rate water measurements are critical to plant control and rating-derating decisions. For this reason, the Electric Power Research Institute (EPRI) has requested NIST assistance in evaluating ultrasonic flow metering technology in a program designed to produce guidelines and standards to improve flow measurements. Concerns for improved standards and guidelines of this type have long been the focus of our research in the Flow Meter Installation Effects (FMIE) consortium.

Approach: The non-ideal flow distributions arising from commonly employed piping arrangements, e.g., elbows, reducers, headers, etc., have been measured as part of our FMIE research effort for a number of years. The FMIE work included measurement of meter performance (typically for orifice and turbine meters) in non-ideal flows and correlation of meter performance to flow characteristics such as swirl and skewness. In the last few years, we have started an investigation of the use of CFD to model these flows and shown, for example, that CFD descriptions of the flow through a conventional pipe elbow agreed favorably with the corresponding laser doppler velocimetry (LDV) measurements. Thus, CFD may afford a reliable method (which can be validated from our FMIE database) for description of the complete flow distributions for a wide variety of industrially important piping geometries. Unfortunately, most conventional flowmeters are far too complex to model accurately the effects of non-ideal flow distributions on meter performance. In contrast, ultrasonic flow metering technology is based on measurement of the time-of-transit of a sound pulse along a given path through the moving fluid. Knowledge of the flow distribution should enable calculation of the meter response, and it is also possible that measurements along multiple paths could, in conjunction with modeling, yield information about flow distributions inside the pipe. This, in effect, affords the potential for in-situ diagnostics of non-ideal installations of great interest to the FMIE consortium. In addition, ultrasonic techniques are of special interest to EPRI because the technology is simple, non-intrusive, and can be installed easily by clamping sensors on the outside of the pipe.

Results and Future Plans: Our CFD efforts have examined flow development in long, straight lengths of pipe to determine the distances required to reach fully developed flow conditions. This study has been done for a Reynolds number of 427,000 where validation data, including turbulence, is available. Different inlet conditions were computed and results showed that the distances required to produce fully-developed profiles ranged between 40 and 90 diameters. Uniform inlet conditions required the shortest lengths; swirled inlet flows produced the longest lengths. These results have guided the development and installation of several types of flow conditioning elements for the 250 mm diameter water-flow test section which will be used for studying the performance of ultrasonic flow meters. We have measured the resulting axial and transverse components of the mean flow profile and found these to be satisfactory (approximately 1-3%) for our purposes. A special pipe section has been designed and built to enable LDV to be used to check the mean and turbulent distributions of the flows for phase 1 testing (see below).

We have also modeled the non-ideal flows associated with the single elbow and the concentric reducer. These have produced flow field results for Reynolds numbers of $3x10^6$ which is the maximum value planned for the meter testing part of our program. These efforts extended our previous flow modeling and LDV validated results done for Reynolds numbers of 100,000 in our FMIE programs.

Using our CFD results, we also have simulated the performance of transit-time ultrasonic flow meters for a wide range of conditions. Firstly, we analyzed the differences between actual ultrasonic paths and the straight line between sending and receiving transmitters. These were found to be dependent upon the flow Mach number and, for all the conditions planned for our EPRI tests, these differences are negligible. Additionally, we analyzed metering errors in fully developed pipe flow conditions and found that up to +5% errors could result if meters were programmed to measure uniform flows. We also found that only a few tenths of percent error occurred as the result of programming the meter to measure any of the well known approximations for the radial flow distributions characteristic of fully developed pipe flow. In simulating meter performance near the exit of the conventional, and prevalent in EPRI practice, single 90° elbow, we found that for typical conditions metering errors ranged from -3% to -15%, depending on the angle of ultrasonic transmission relative to the plane of symmetry of the elbow. This result has already been helpful to EPRI personnel because they were adopting the practice of installing clamp-on meters at the orientation which produced the average value of the two extremes found from multiple meter installations. Their assumption had been that, since in-situ calibrations were not feasible, the extreme values probably had relative errors that were the same in magnitude and opposite in sign, and therefore the average was a good approximation of the correct value. Our results show that this is not so. More needs to be known about these flows and more technology needs to be added to these techniques to improve metering practice.

Our NIST-EPRI efforts in the past year also included convening a workshop of utility industry personnel, ultrasonic flow meter manufacturers, and other flow meter users to discuss and design the tests needed for efficient and effective progress toward our goals of improved ultrasonic metering and improved standards of practice. The tests will be done on commercially available meters installed by their manufacturers to attain their ultimate capabilities in the three phases of testing. The first phase will be done in ideal conditions; the second and third will test these same meters downstream of the single elbow and the concentric reducer, respectively. This test plan is designed to produce the database with which to assess this technology for its use as: (1) a replacement for existing flow meters, (2) an in-situ calibration capability for installed meters, or (3) a diagnostic tool for assessing the characteristics of pipe flows entering existing meters. To initiate our NIST-EPRI program, we have in the last year produced a CRADA for the meter manufacturers interested in participating in our program; this agreement is being reviewed and five meter manufacturers are expected to participate. Ultrasonic meter test results are expected in FY 1997.

Publication:

Yeh, T.T. and Mattingly, G.E., "**Flow Meter Installation Effects Due to a Generic Header**," NIST Technical Note 1419 (1996).

9. *Exhaust Meter Calibration Facility*

***J.D. Wright**, P.I. Espina, F.I. Padilla, and G.E. Mattingly*

Objective: To establish an Exhaust Meter Calibration Facility (EMCF) that generates a simulated car exhaust gas flow known to a expanded uncertainty of 1%. The EMCF will test the performance of exhaust flow meters under the demanding conditions of widely varying flow, temperature, and composition found during actual vehicle testing.

Problem: Auto manufacturers are working to develop ultra-low emission vehicles and meet clean air requirements. Critical tools in these efforts are flow meters to accurately measure the flow of exhaust. The American Industry / Government Emissions Research group (AIGER) requested a facility in which exhaust flow meters can be performance tested and calibrated. There is currently no facility available which can generate flows with accurately known, yet widely variable, composition and with the extreme range of temperature and flow found in exhaust. Also, the methodologies for extending calibrations made under easily attained conditions, i.e., room temperature and dry air composition, to the extreme conditions of real meter applications have not been thoroughly validated for the various meter types in use.

Approach: The EMCF will test flow meters under the conditions seen in actual vehicle testing by 1) accurately mixing component gases to simulate vehicle exhaust, 2) varying the exhaust temperature between 290 K and 700 K, and 3) measuring the combined flow to better than 1% of reading. The flow range for the simulated exhaust will be 0.041 $\mathrm{mol \cdot s^{-1}}$ to 1.48 $\mathrm{mol \cdot s^{-1}}$ while humidified air flows supported by the facility will range from 0.041 $\mathrm{mol \cdot s^{-1}}$ to 4.1 $\mathrm{mol \cdot s^{-1}}$. Each of the component gas streams, nitrogen, carbon dioxide, and argon will be metered with a bank of three critical flow nozzles sized to cover the broad range of flows. The nitrogen gas stream will be preheated, then passed through a humidifier vessel to introduce the desired flow of water vapor, and the water flow will be measured using an optical hygrometer. The mixed gas stream will be heated to the set point temperature by a large electric circulation heater before entering the meter under test. The facility will have a computer data acquisition and control system which will allow the automated collection of calibration data at pre-determined set points of composition, temperature, and flow.

The completed facility will be a significant contribution to the study of the effects of gas composition and temperature on flow meter performance. It will allow users to test the validity of the extrapolation of meter calibration data (via the appropriate dimensionless quantities for the particular metering method) to extremes of composition and temperature that are beyond the capability of existing calibration facilities. The EMCF will also be available for flow meter manufacturers working to develop meters that are immune to changes in composition and temperature.

Results and Future Plans: In the past year the EMCF was completed and was demonstrated to the AIGER group in August. Since that time, the facility has been in constant use by NIST to prove and quantify its performance capabilities relative to design goals, and by AIGER members to initiate testing of their exhaust flow meters. The EMCF has been operated over its full flow range and its measurement performance exceeds the uncertainty specifications of the design for conditions near ambient. Operation at elevated temperatures, with multiple gas sources, and at high moisture content all have been successful, but significant effort has been devoted to numerous improvements in individual sensing systems, hardware adjustments, and in set point and procedure sequences. Improved software features have reduced the times required to reach set points and check for stability after temperature, composition, or flow rate changes are made. The collaborations with AIGER members have produced the preliminary assessments needed to quantify current limitations of the flow meters that they are evaluating for exhaust flow measurement. These results are benefitting both the AIGER members and the flow meter manufacturers by guiding both of these groups in their efforts to improve meter performance.

Future plans include efforts to prove the repeatability and stability of the EMCF, to further reduce equilibration times, and to validate its performance using our NIST reference standards for air flow. We plan to collaborate with AIGER members to develop and validate methodologies for attaining satisfactory results in real exhaust measurement applications. As well, we plan to incorporate the involvements and capabilities of the flow meter manufacturers into these programs to further enhance our collective progress.

In the coming year we also plan to use the EMCF to develop a new NIST calibration service for gas flow rate with variable composition and temperature. This effort would produce and then advertise the pertinent expanded uncertainty that we can attain for gas flow having the above-stated ranges of conditions.

10. Characterization and Optimization of Residual Gas Analyzers for Semiconductor Processing

C.R. Tilford, M. Li (College of William and Mary), and S.A. Tison

Objective: To characterize and improve the metrological properties of residual or partial pressure analyzers (RGAs or PPAs), and develop *in situ* calibration techniques for processing applications.

Problem: Residual gas analyzers have been widely used for qualitative vacuum system diagnostics. The increasing complexity of semiconductor processing has generated a further need for real-time process monitoring, typically to monitor gaseous contaminants in the 10^{-7} Pa to 10^{-4} Pa range or for process control. Both of these require quantitative measurements if they are to be successful in a large-scale manufacturing environment. However, to the extent that they are known, the quantitative capabilities of many (most) RGAs are not adequate for these tasks. There is a need to better understand these instruments, improve their performance, and insure consistent results by providing the users with access to national standards.

Approach: Obtain quantitative test data for commercial RGAs and develop generic RGA operation models that allow determination of critical performance parameters, optimum operational conditions, and instrument design improvements. Transfer NIST vacuum standards capabilities to users through *in situ* calibration techniques.

Results and Future Plans: Experimental data and analytic modeling completed this past year strongly indicate that the more undesirable performance characteristics of RGAs are caused by the effects of electron and ion space charge throughout the instrument. While the evidence is not complete, the preponderance of the evidence is that the severe nonlinearities with pressure, and the dependence of the sensitivity for one species on the pressure of other species, are caused by a pressure dependent combination of three distinct space charge effects.

The first of these effects is a competition between electron and ion space charges in the ion source. The electron space charge decreases the ion energy and the transmission efficiency of the quadrupole mass filter, in some cases by as much as two orders of magnitude. As the total pressure increases there is a corresponding increase of ion space charge that counteracts the (approximately constant) electron space charge, with a corresponding increase in the ion energy, transmission efficiency, and instrument sensitivity. This model had been previously proposed; the present data are the only direct evidence that it works for most instruments.

With further increases in pressure (and thus ion current) the sensitivity change is reversed to a rapid decrease with increasing pressure. Although previously thought due to ion-neutral collisions, modeling completed this past year predicts that the sensitivity decrease is due to expansion of the ion beam, a space charge effect caused by self-repulsion of the ions, in the region between the ion source and mass filter. A series of measurements with different gases validates this explanation. At the highest pressures (and ion currents), generally not encountered in typically applications of RGAs, but quite common in semiconductor processing, a similar beam expansion occuring within the mass filter should cause further reductions in sensitivity. Again, the experimental evidence supports this explanation.

Fortunately, space-charge effects can be minimized for most instruments which will have the effect of reducing the unwanted non-linearities. Decreasing the electron emission current directly reduces the perturbation from electron space charge of ion energy within the ion source. Further, reduction of electron emission current causes a corresponding decrease in the ion current, and therefore, the ion space charge throughout the instrument. Increasing the ion energy (at fixed ion current) also leads to reduction of the ion space charge density. The model also predicts that reducing the distance between the ion source and the mass filter will minimize beam expansion in that region and extend the linear range of the RGA to higher pressures. Performance data for a new small-geometry RGA indicate an increased linear range, by at least an order of magnitude, consistent with the model's predictions.

Our understanding of RGAs is far from complete, and we plan further experiments to refine our understanding of high-pressure performance. We also plan to apply some of the concepts that have worked so well at high pressures to find the causes of low-pressure nonlinearities observed in some instruments. However, in spite of these limitations, we now feel that the performance that we can obtain from many RGAs is adequate to warrant calibrating the instrument. We hope to test both our RGA optimization and our calibration techniques in a university facility that is exploring the use of RGAs for realtime semiconductor process control.

Publications:

Tilford, C.R., "**Gas Contamination Monitoring for Semiconductor Procesing,**" in Semiconductor Characterization: Present Status and Future Needs, ed. W.M. Bullis, D.G. Seiler and A.C. Diebold, AIP Conference Proceedings, 252 (1996).

11. Measurement and Standards for the Metering of Low-Gas Flows

S.A. Tison** and **C.R. Tilford

Objective: To develop primary flow standards in the range of (10^{-7} to 10^{-3}) mol/s and transfer this flow measurement capability to U.S. industry, government, and academic institutions.

Problem: Many industrial processes require the accurate metering of mass flow over the range of 10^{-7} to 10^{-3} mol/s. In particuar, the SEMI/SEMATECH Mass Flow Controller working group identified the need to measure flows in this range with total expanded uncertainties of 1% or less and identified the need for national flow standards in the range with total expanded uncertainties of 0.2% or less. National standards for flow in this range are not adequate to meet the identified industrial requirements.

Approach: The program will develop primary flow standards which would operate over a range of (10^{-7} to 10^{-3}) mol/s with expanded uncertainties of 0.1% or less. To transfer this measurement capability to industrial users the properties of commercially available flow meters will be investigated and the development of highly repeatable flow meters will be explored.

Results and Future Plans: A constant volume primary flow meter was constructed which operates over a range of (10^{-9} to 10^{-3}) mol/s with expanded uncertainties of 0.1% or less. This system utilizes two accumulation volumes, 2 liters and 150 liters, in conjunction with high accuracy pressure and temperature instrumentation to calculate the change in mass in the volume when a flow is introduced. The system is of all metal construction with high-vacuum type fittings and is compatible with all non-corrosive gases. This system was compared with existing flow standards in the Pressure and Vacuum Group over their overlapping ranges of (10^{-8} to 5×10^{-6}) mol/s. The two standards agreed to within their respective uncertainties of ±0.1%. The system was also compared with flow standards in the Fluid Flow Group over the range of (10^{-4} to 10^{-3}) mol/s. The systems agreed to within their expanded standard uncertainties.

A series of ultra-stable laminar flow meters have been developed which use pressure differentials as large as 0.8 MPa to generate flows over a range of (10^{-7} to 10^{-3}) mol/s. These instruments have demonstrated long term stabilities over the course of one year of ±0.1% (relative expanded uncertainty). A number of companies have shown interest in commercializing the NIST developed laminar flow meter. These flow meters have been used by NIST personnel to conduct on-site proficiency test with industrial primary flow standards at five companies during FY 1996. The results of the proficiency tests with nitrogen reveal that the tested industrial standards were within their specified uncertainties for flows larger than 10^{-4} mol/s but many were significantly outside of their estimated uncertainties for lower flows. Proficiency testing with other gases such as sulfur hexafluoride, argon, and helium indicated greater measurement deficiencies. In principle, the tested primary standards were capable of operating at the same level of uncertainty for any non-reactive gas but the data reveal that a number of the industrial standards were operating outside of their estimated uncertainties when operated with argon or sulfur hexafluoride.

In FY 1997 a constant pressure flowmeter that operates over a range of (10^{-7} to 10^{-3}) mols/s will be developed. Alternate techniques including ultrasonic flow metering are being developed to address issues concerning measurement of toxic or corrosive gases. Collaborations with industrial consortia such as SEMI/SEMATECH to transfer the new NIST flow measurement capability to U.S. industry will be pursued.

Publications:

Tison, S.A., "**Improved Gas Flow Measurements for Next-Generation Processes,**" in Semiconductor Characterization: Present Status and Future Needs, ed. W.M. Bullis, D.G. Seiler and A.C. Diebold, AIP Conference Proceedings, 497 (1996).

Tison, S.A., "**A Critical Evaluation of Thermal Mass Flow Meters** ," J. Vac. Sci. Technol. A 14(4), 2582 (1996).

Tison, S.A., "**Accurate Flow Measurement in Vacuum Processing,**" Solid State Technology, Oct., 73 (1996).

12. Models and Data for Semiconductor Processing

M.R. Zachariah, D.R. Burgess, Jr., M.J. Carrier, R. Davis, E. Moore, J. Maslar, K. Steffens, W. Tsang (833), O. Sanogo, C.F. Melius (Sandia Laboratories), R. Berry (Wright-Patterson Laboratories), and M. Kushner (Univ. of Illinois)

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

1. *Ab-initio* quantum chemistry and reaction rate theory derived thermochemical and kinetic data for silicon based process chemistries.
2. Development of user friendly data handling procedures and data estimation software.
3. Develop software capable of modeling flow, chemistry and particle contamination in prototypical thermal chemical vapor deposition (CVD) reactors.
4. Benchmarking of data and models against selected experiments.

Problem: Reactor and process design are currently limited to empirical trial and error approaches which tend to converge to semi-optimized states very slowly. This implies that for an industry where changes occur rapidly, processes are not adequately investigated prior to final implementation. This effect manifests itself not only in more costly, possibly poorer quality products, but processes which may be environmentally dirtier.

Approach: Process simulation has the potential to make the design phase of process development considerably more efficient and therefore, both more effective and more likely to have an impact on the final process, given the typically severe time constraints. This potential has been enabled by ever-increasing computational power which has evolved to the point that very sophisticated models can be constructed for a variety of complex semiconductor processes. However, increasing complexity of models implies greater need for fundamental thermochemical and kinetic data which are not readily available. Our approach is both to use and develop methods for reliably generating, from first principles, thermochemical and kinetic data suitable for process modeling. In some cases, methods will involve development of user friendly tools that will aid an experienced process engineer in generating the data necessary for modeling as well as methods for efficient post-processing of simulation results. Furthermore, methods for handling, collating, and disseminating the information in an efficient and timely manner are an important component to the task. Finally the reliability and utility of data generated in such a manner is both a function of the inherent quality of the data (which must be verified) and the demonstration of its utility to the user community. The latter point requires that prototypical models be developed for which benchmarking studies can conducted.

Results and Future Plans: This year we have continued our development of thermodynamic and kinetic data through the application of ab-initio molecular orbital methods and reaction rate theory. Data have been generated on the thermochemistry of silicon-phosphorus-hydrogen system as well as some selected transition states. The motivation for the work is to enable development of a predictive model for silicon film growth under phosphorus dopant addition. Currently there is no such model and thus no explanation for the experimentally-observed 20-fold increase in film growth rate found with the addition of PH_3.

As part of our effort to benchmark chemistries important in silicon processing, we have undertaken high temperature kinetic and mechanistic studies for tetraethoxysilane (TEOS) decomposition. This is the primary precursor used in both thermal and plasma enhanced CVD growth of silicon dioxide films. This year we have determined the temperature dependent elementary rate constant for the reaction of TEOS with oxygen atoms and are developing a qualitative mechanistic view of TEOS oxidation. This is a key issue for on-going modeling efforts of silicon processing.

One of the significant problems faced by the semiconductor industry is the environmental impact of semiconductor processing, in particular its heavy use of chlorofluorocarbons for plasma etching and chamber cleaning. These processes result in effluents from reactors that need some type of remediation. This year we have developed a novel approach for the destruction of chlorofluorocarbons (CFCs). Sodium vapor is mixed with vapor phase CFCs resulting in rapid mineralization into sodium halide salts and elemental carbon aerosols. The advantage of the approach is the reduction of the halogenated organic to a nonvolatile and nontoxic product.

We continue our efforts in the use of laser induced fluorescence imaging of fluorocarbon etching plasmas in the gaseous electronics conference (GEC) plasma cell to characterize in 2-dimensions the chemical speciation. We are currently monitoring CF_2 as a signature species in the plasma in an effort to provide data suitable for validation of 2-D plasma models. This year, in collaboration with Professor Mark Kushner of the University of Illinois, modeling of some of our previous results on Ar metastable distributions has been initiated. The preliminary results are very encouraging as the general features seen in our experiments have been reproduced with the model. Future effort will focus on the fluorocarbon chemistry and the modeling of particle transport within the plasma cell. In our experimental program, we are investigating the effect of precursor and elemental composition on plasma chemistry. In these experiments, CF_2 is monitored as a function of elemental composition (C:H:F ratio) and of precursor form (as, or combinations of, CF_4, CHF_3, CH_2F_2, CH_3F, CH_4). We monitored the formation of particulates within the plasma, since this is a very important issue for quality control in wafer processing. Our results indicate that the CF_2 concentration is only mildly sensitive to the nature of the precursor but very sensitive to the balance of elemental components. On the other hand, particle formation was found to be sensitive to both the elemental of balance and precursor form. Future efforts will be aimed at quantifying the formation of particulates and their movement within the plasma.

Fabrication, assembly, testing, and calibration of a rotating-polarizer, spectroscopic ellipsometer (SE) have been completed this year. The SE is designed for both *in situ* and *ex situ* measurements of the thickness and chemical composition of surface layers produced in either the GEC Reference Cell or the rotating disk thermal CVD reference reactor (see below). Special optical arrangements to allow *in situ* measurements also have been designed for both reactors. The SE is fully automated, including analyzer azimuth setting, polarizer rotational frequency control, and system calibration. This new capability will allow correlation of observed gas phase chemical and electrical conditions with surface film growth and composition.

Work on the development of a numerical model for micro-contamination in a rotating disk CVD reactor continues, with a focus on chemical kinetics and transport. A 60-step reaction mechanism which simulates both silane decomposition and particle nucleation has been incorporated into the model. The process of species condensation on particles leaving the reactor has been incorporated and found to be one of the dominant modes of particle growth. Reactor performance maps have been generated which indicate the temperature regimes where surface growth and contamination rates are each within acceptable levels. Lower reactor pressure and higher disk rotation rate also have been found to be beneficial. We anticipate performing the first comparisons with experimental data in our reference CVD reactor during FY 1997.

This year we have also developed a WWW accessible, interactive database for chemical kinetic mechanisms called Web CKMech. It can be found through the group home page at "http://fluid.nist.gov:80/836.03". The interactive database was developed to make our prior compilations and computations (many of them computed by *ab-initio* methods) of fluorocarbons thermochemistry and kinetics more accessible. Data on CKMech can be searched by chemical formula or compound class, and will provide thermochemical functions, NASA thermochemical polynomial fits, molecular geometry data (bond distances, angles), moments of inertia, vibrational frequencies, and molecular transport properties. It also provides the output from *ab-initio* computations (HF geometries, MP4 energies). The data can also be searched by reactions to provide rate coefficients for specifies reactions as well as full bibliographic information.

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13. Measurements and Models for Plasma Processing of Semiconductors

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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 a long term objective of the semiconductor industry, reflected in the Semiconductor Industry Association's 1994 National Technology Roadmap for Semiconductors. Significant advances in low-temperature plasma equipment modeling have occurred in the last decade. Nevertheless, in a number of specific areas, further progress in model development and validation is required. Relatively little effort has been made to model the electrical behavior of processing reactors and plasmas contained inside them, even though electrical factors can be critical to ensuring optimal and repeatable process results. This neglect is partly due to a lack of experimental data, and partly due to the computational difficulties imposed by the asymmetry of commercial reactors and by the narrow sheath regions that form between the plasma and the reactor surfaces. Another important limitation of plasma modeling is that many of the cross sections needed to model the chemistry of the plasma are unknown or not known to sufficient accuracy. Finally, because most plasma diagnostic techniques are incompatible with the manufacturing environment, a need exists to develop sensors for that environment, models to interpret the sensor readings, and new schemes of closed-loop control based on these sensors and models.

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 cells), provide a well-defined basis for comparison of measurements between laboratories. The cells are equipped with a wide variety of plasma diagnostic tools that measure the chemical, physical, and electrical properties of the plasma, and the relations between these properties. 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. For such applications, radio-frequency current and voltage measurements show great promise since they may be performed externally, without perturbing the plasma or contaminating the wafers. These electrical measurements contain information about plasma properties relevant to processing, such as the flux and kinetic energy distributions of ions, and the sheath width, sheath voltage, and sheath electric field. Modeling efforts have emphasized the relations between these physical plasma properties and the electrical measurements. The models can be used both to develop electrical sensors for plasma properties and to optimize the electrical behavior of plasma processes and equipment.

Results and Future Plans: Radio-frequency electrical measurements and models have been applied to characterize fluorinated gas plasmas used for in situ cleaning of plasma-enhanced chemical vapor deposition reactors. Measurements were made for Ar/NF_3, $Ar/CF_4/O_2$, and $Ar/C_2F_6/O_2$ plasmas in a GEC Reference Cell, located at Air Products and Chemicals, Inc. The goal of this study was to identify the plasma conditions that produce optimal reactor cleaning, and to determine more efficient means of setting process control variables to achieve optimal results. The achievement of rapid etching rates, necessary for optimal processing, was found to be critically dependent on electrical factors. Previously observed local maxima in etch rate, at intermediate pressures and mixture ratios, were explained by measurements of the power losses in the plasma itself and in the surrounding circuitry. The electrical behavior of the plasma was similar for all gas mixtures studied, and it can be represented by a simple and general equivalent circuit model. The model is general enough to apply to any reactor geometry. Use of the model, combined with the RF electrical measurements, should greatly simplify the task of finding and maintaining optimal reactor cleaning procedures, even in reactors quite different from the GEC cell. The model may also be used to optimize the design of the reactors themselves and the circuitry that powers them. This work also provides a firmer foundation and a better-defined basis of comparison for studies of plasma chemistry issues, such as the need to reduce the greenhouse potential of plasma effluents.

Also this year, the ion flux at the powered electrode of a GEC cell at NIST was measured by simultaneously imposing dc and rf voltages on the electrode. Measurements of the impedance of the sheath at the powered electrode were combined with ion flux measurements to test models of the electrical behavior of radio-frequency plasma sheaths. The data were in good but not perfect agreement with sheath models derived for symmetric discharges. A new sheath model designed for the sheath at the powered electrode of highly asymmetric discharges was found to be in even better agreement with experiment. The validation of the sheath models proved useful in the analysis of chamber cleaning plasmas described above. The ion flux measurement technique that we implemented is not suitable for industrial applications where a thin insulating layer may be present on the wafer being processed. Nevertheless, this work suggests that rf measurements, interpreted using a validated sheath model, may serve as the basis of a practical ion flux sensor for industrial applications.

The trend in state-of-the-art wafer processing is toward higher density RF plasma sources and lower operating pressures to increase process rates and reduce contamination. Current reactor designs favor inductively-coupled sources. The role of ion chemistry and the plasma sheaths in these reactors differ from capacitively-coupled reactors. Experiments to study the electrical properties of the high-density sheaths formed in an inductively-coupled GEC cell were begun this year and will continue in the future. Models describing the electrical behavior of the high density sheaths will be developed. Studies are also planned to evaluate the relative importance of ion and neutral radical reactions at the low pressures used in high-density reactors.

The semiconductor industry is also shifting to 300 mm single-wafer processing which introduces additional challenges including the need to maintain spatial uniformity over a wider area. Preliminary designs have been drawn on a new research reactor to be constructed based on the 300 mm scale and intended to mimic industrial high-density plasma reactors. Experiments are planned to investigate chemical and electrical uniformity issues in this reactor and to identify the underlying chemical reactions critical to etching, damage mechanisms, and process efficiency.

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14. Gas Sensing with Micro-Hotplate Sensor Arrays

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Objective: To demonstrate feasibility and manufacturability of silicon micro-machined, thin-film sensor array technology for multi-analyte, real-time detection and concentration measurement of gas phase species.

Problem: Detection and measurement of individual and multiple gas phase species are currently achieved with relatively expensive, ruggedized analytical instruments. Response times vary from minutes to several hours. Inexpensive, real-time or near real-time measurement capability with the required sensitivity can provide a sensing and measurement technology of broad utility, e.g., process control, chemical identification/verification of materials, auto emission measurement, and environmental monitoring.

Approach: Micro-hotplate chemical sensor arrays are monolithic, CMOS-fabricated, bridge structures formed with post-CMOS process silicon micro-machining. These multi-layer structures were designed and have been patented by NIST. They have three functional layers, a heater, a thermometer/heat distribution plate, and sensing film electrical contacts. These are easily fabricated in an array format with typical single element sizes that range from (100 to 250) µm. Device fabrication takes advantage of commercially available CMOS processing, thereby providing a path to a manufacturable sensing technology. Change in electrical conductance of semiconducting oxide thin films, e.g., SnO_2, ZnO, and TiO_2, is the transduction method for detection of chemical species adsorbed on the film surface. These sensor structures are sensitive and selective, capable of real-time monitoring, low cost, and robust. Surface dispersed, catalytic metal additives can alter response sensitivity and selectivity of each array element's sensing film.

Micro-hotplates have low power requirements, i.e., tens of milliwatts, large operating temperature range (>800°C), and heating time constants of 1 - 2 milliseconds. This capability supports a novel sensing approach, temperature programmed sensing, (TPS). TPS on the sub-second scale and in the array context has excellent potential as a chemical species measurement technique for real-time sensing of individual species in multi-component gas mixtures. TPS response signatures are characteristic of adsorbed species/sensing material combinations. Algorithm development is an important aspect of the research effort.

The project requires an interdisciplinary approach with expertise in the areas of metal oxide sensing materials, their deposition as thin films, deposition of surface dispersed metal additives, IC design and fabrication, silicon surface micro-machining, and analysis of signal response patterns.

Results and Future Plans: Response data for single chemical species in air have been obtained for a number of gas species including CO, CO_2, ethanol, methanol, propanol, acetone, and perchloroethylene. Extensive data sets have been obtained for CO in air, ethanol, and methanol. These demonstrate both detection limits and response linearity. For CO in air, detection limits have been demonstrated at the 2 - 3 mg/kg level with good linearity up to 1000 mg/kg. Testing of individual micro-hotplates for approximately one year demonstrate good sensitivity and reproducibility of TPS response patterns.

TPS response pattern recognition algorithms are the means to detect and measure concentrations of single and multiple species. An optimization method has been developed that demonstrates the capability to differentiate between species having very similar response characteristics. Differentiation between quite dissimilar response patterns is also achieved.

Deposition of different oxides on individual elements of a 4-element array has been demonstrated for two semiconducting metal oxides, ZnO and SnO_2, and one catalytic metal, Pt, using the chemical vapor deposition (CVD) process. This is a self-aligned process taking advantage of the ability to individually heat individual array elements. The dependence of film morphology on deposition parameters has been investigated. These studies have been significantly accelerated by the use of advanced CMOS processing that allow substitution of a tungsten-titanium alloy for aluminum in the metal layers. This has resulted in much more robust sensing-film electrical contacts.

Continued technological and scientific developments are being pursued to expand the number of sensing materials and catalytic metal surface additives and to investigate sensing-film formation characteristics and morphologies and their effects on sensing response. Investigation of growth and relation to sensing response characteristics continues. WO_3, TiO_2, Pd, Ni, Fe, or Cu will be investigated. Investigation of individual and array response characteristics to mixtures of organics gases rely on continued development of both response recognition algorithms and on a broader range of sensing film materials.

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15. Self-Assembled Monolayers for Biosensing

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Objective: To develop and apply characterization methods sensitive to physical and chemical surface properties of single and multi-layer structures to determine structural features and to demonstrate the occurrence of molecular recognition events. Self-assembled monolayers (SAMs) are used as model systems to develop fundamental, quantitative knowledge of molecular recognition structures and reactions.

Problem: Future biosensors will be required to perform multi-analyte measurements rapidly, accurately, and at increasingly lower cost. Surface confined arrays of highly-selective sensing elements are promising in this regard. For example, the use of surface-bound single-stranded DNA (ssDNA) probes in array-based devices for genetic screening and sequencing has generated considerable interest and excitement in the biotechnology community. In spite of the promise of this technology, little is know about the optimal surface conditions for promoting hybridization of the surface-bound probes. In particular, the surface conformations and optimal probe surface coverage for maximizing hybridization have not been studied in detail. To achieve an improved level of understanding, characterization methods are necessary for elucidating both the structural properties of biomolecular layers and their molecular recognition reactions.

Approach: Alkanethiol SAMs impart chemical sensitivity to Au surfaces, forming robust, ordered structures with highly tunable surface characteristics. The thiol functionality bonds strongly to the gold surface, forming a densely-packed monolayer with the opposite ends of the thiol molecule forming the SAM surface. Fully-functional biological monolayers (protein or nucleic acid) can be prepared by derivatizing the biological molecule of interest with a thiol group, and then self-assembling the thiol-derivatized biomolecule on Au. Our studies have focussed on the surface characterization of thiol-derivatized, ssDNA monolayers. The self-assembled DNA monolayers can be easily prepared by exposing clean, bare Au to an aqueous solution of thiol-derivatized DNA.

Results and Future Plans: The surface density, hybridization activity, molecular orientation, and monolayer thickness of the surface-bound DNA probes have been characterized with an extensive range of surface-sensitive techniques. These include x-ray photoelectron spectroscopy, grazing angle Fourier transform infrared spectroscopy, surface-enhanced Raman spectroscopy, ellipsometry, scanning tunneling microscopy (STM), secondary ion mass spectrometry (SIMS), ^{32}P radio labeling, and surface plasmon resonance (SPR) spectroscopy. Using combinations of these methods, a process for immobilizing ssDNA on Au with precisely-controlled surface coverage has been demonstrated. This process provides a high degree of control over DNA probe surface coverage by forming a two-component monolayer of the thiol-derivatized ssDNA with a diluent thiol, mercaptohexanol (MCH). Non-specifically bound ssDNA is effectively removed such that thiolated ssDNA probes are fully active and available to participate in molecular recognition events. To monitor hybridization reactions of surface-bound ssDNA prepared in this way, ^{32}P radio labeling has been used to demonstrate that up to 50% of the surface immobilized ssDNA hybridized with complementary DNA targets. This is a much higher hybridization efficiency than reported previously. Additionally, hybridization reactions between surface-bound ssDNA and its complement are dependent on probe molecule surface coverage. A patent application for this process has been submitted.

To demonstrate the high selectivity of molecular recognition reactions and the robust nature of surface-bound ssDNA, surface immobilized ssDNA was hybridized with its target strand. The duplex was then melted, leaving only the ssDNA on the surface. This DNA-derivatized surface retained its specificity in subsequent hybridization reactions, binding again (and only) with complementary ssDNA.

In preliminary studies, SPR has been used to monitor and quantify ssDNA probe coverage and to follow both the kinetics of hybridization and the process of thermally induced dehybridization. The SPR technique provides *in situ* hybridization detection, not requiring fluorescent or radioactive tags, thereby simplifying detection procedures.

High resolution STM imaging of mercaptohexanol SAMs formed by vapor phase dosing has been used to study the self-assembly mechanism on single crystal gold surfaces. Series of molecular-scale images taken as the dosing occurs provide a detailed picture of the self-assembly mechanism of alkanethiols and the direct observation of the nucleation, growth, and phase transitions of these molecular layers in real-time. Although many techniques have been used to study the equilibrium structure of such layers, the mechanism of self-assembly has largely been unknown until this work was completed.

Current and future plans include a detailed investigation of the effect of hybridization solution conditions, e.g., pH, buffer strength and identity, on the hybridization efficiency and stability of the surface-immobilized probes. In addition, we plan to examine the applicability of vibrational spectroscopies (infrared and Raman) for characterizing ssDNA on metal surfaces, and for monitoring hybridization reactions of surface-bound DNA.

Publications:

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16. Synthesis and Characterization of Nanostructured Materials

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Objective: To development of a knowledge base for vapor-phase processing of bulk quantities of nanostructured materials.

1) Characterization and modeling of sodium/metal-halide based chemistry for production of unagglomerated metals and ceramics.
2) Development of prototypical methods for vapor phase production of nanocomposites.

Problem: Various theoretical and experimental results have shown that materials can demonstrate new and enhanced properties (mechanical, optical, electrical, etc.) when constructed from components whose composition and size has been modulated on nanometer dimensions. However, methods to grow such materials reliably with tunable composition, size, and morphology need development. In addition, the knowledge base necessary to design and control the chemical/physical processes inherent in vapor phase gas-solid transformations need further investigation.

Approach: Our approach has been to use methods for synthesis of nanostructured materials in particulate form, with an emphasis on methods that have the potential to be extended to the production of materials on a bulk scale. The vapor-phase route has been pursued as potentially the most robust generic method to supply the basic requirements for cost-effectiveness in manufacture and the resulting material property. Two synergistic approaches are being pursued. 1) Development of new methods and chemistries for material production, and investigation of their resulting performance. 2) Application of advanced *in situ* diagnostics for characterization of these processes and development and application of molecular-level models for prediction of nucleation and growth of nanoscale materials.

Results and Future Plans: The primary activity this year has been the implementation of a gas phase sodium reduction process for the production of non-oxide particles. The basic chemistry for the process involves the reaction of sodium vapor with metal halides (**$x\mathrm{Na} + \mathrm{MCl}_x \Rightarrow \mathrm{M} + x\mathrm{NaCl}$**), which results in the condensation of small metal clusters encapsulated in salt. The chemistry is generic and should be applicable to the synthesis of a wide class of materials (metals, intermetallics, ceramics). The potential strength of the process is not only its generic chemistry, but the fact that a by-product of the reaction, NaCl, serves as a coating to the particles, preventing agglomeration. Furthermore, the synthesis scheme can clearly be classified as "green", as no toxic by-products are involved. We have completed a study of the (dual-source) reaction kinetics for Ti, B, and TiB_2 chemistry which has indicated that the chemistry is very fast and amenable to a turbulent flow environment with the possible benefit of high volume production. We will investigate the kinetics of particle growth at low pressures.

We also have been studying the effect of precursor (single and dual-source) on the formation of SiC (a high-temperature, -strength, -durability ceramic) with the goal of producing nanometer-scale material in unagglomerated form. Our results suggest that unlike the Ti/B chemistry, dual-source precursors lead to separate reaction zones and the formation of Si/SiC composites. Use of a single-source precursor, methyltrichlorosilane, also produced Si/SiC which we have determined is due to thermal decomposition of the first reaction product of the precursor, leading to a volatile hydrocarbon (loss of carbon). The problem of carbon loss has been surmounted by addition of excess carbon in the form of CF_3Cl. With this addition, we can indeed produce phase-pure, nanostructured SiC in a relatively unagglomerated state. Our current efforts are aimed at developing a more thorough elucidation of this chemistry and the general nature of the interaction of unsaturated hydrocarbons with sodium vapor. Future efforts also will be directed to understanding and controlling the formation of the salt coating and its relationship to agglomeration and particle growth.

This year we published a thorough study of the properties of silicon nanoparticles by molecular dynamics simulation. The size and temperature dependence of the cluster energy, internal structure, diffusion coefficients, phonon density, and a number of other properties have been revealed. Our current efforts are focused on cluster reactivity and the development of a phenomenological model for particle sintering based on the molecular dynamics computations.

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17. *Measurement Technology for Benchmark Spray Combustion Data*

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Objective: Develop measurement technology to provide benchmark experimental data for input to, and validation of, multiphase combustion models. Establish correlations between operating conditions, and the resultant spray flame characteristics, thermal gradients, and level of chemical byproducts.

Problem: Control of process efficiency and the formation of species byproducts from industrial thermal oxidation systems (*e.g.*, power generation and treatment of process liquid wastes), is generally based on *a priori* knowledge of the input stream global physical and chemical properties, desired stoichiometric conditions, and monitoring of a few major chemical species in the exhaust. Optimization of the performance of these systems relies increasingly on computational models and simulations that help provide relevant process information in a cost-effective manner. In general, 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 reactor region is requires a better knowledge base to enable optimization of the chemical and thermal processes and control of particulate and gaseous emissions. System performance is dependent strongly on the quality of liquid atomization, aerodynamic design, and the degree of liquid/air mixing. The need to provide *in-situ*, real-time data on the characteristics of the droplet field, and its interrelationship with the system operating conditions (*e.g.*, desired stoichiometry), heat transfer, and particulate/gaseous byproducts is crucial for the development of advanced computational models, and the efficient operation of thermal oxidation systems.

Approach: The NIST spray combustion facility (SCF) has evolved into a well-characterized and controlled system which can handle different process liquid fuels and wastes, atomizer designs, and combustor configurations. A variety of diagnostic techniques are being employed to the input fuel stream (fuel composition), spray flame (droplet size, velocity, and temperature), and flame emissions (particulates and chemical species). The measurements are used to provide reliable benchmark data for process optimization and control, real-time *in-situ* sensor development, and input/validation of numerical simulations. A coupled experimental/computational approach is also being implemented by involving modelers from our industrial partners directly in the formulation of the experimental program.

Results and Future Plans: In FY 1996, efforts focused on examination of state-of-the-art liquid fuel injection concepts. Fuel atomization is essential to enhance process performance and control the formation of chemical byproducts. Currently, two atomizer concepts are under investigation, namely: 1) acoustic atomization (with Fluid Jets Associates), and 2) gas/steam-assisted atomization (with the Navy and University of Maryland). In our collaboration with Fluid Jets Associates, a commercially available pressure-jet nozzle, modified with a piezoelectric driver, was used to sustain a kerosene flame in our spray combustion facility. Acoustically driven atomizers are of interest because of the dynamic control that one may achieve of the spray structure. The spray was characterized for two different resonant frequencies (9.0 and 11.8 kHz) under burning conditions. Evaluation of the results indicate that modulation of the fuel stream at high power levels dramatically altered the spray structure and global features of the flame. Large droplets, normally found to pass ballistically outward through the flame sheet, were redirected along the central region of the flame resulting in more complete droplet vaporization.

In thermal oxidation facilities, steam generation is a financially important byproduct that is generally used to provide electrical power. However, some of the steam can be used for other purposes such as to assist in the atomization of the fuel or liquid chemical wastes. We have undertaken an effort to characterize the advantages of steam-assisted atomization and its effect on process efficiencies and species emissions. In order to carry out this task, criteria were developed to enable comparison of the steam results under different operating conditions. Thus, various atomization gases (CO_2, N_2, Ar, He, and O_2) were successfully tested for the first time to isolate the physical and chemical effects on droplet atomization. The results indicated that the spray characteristics should be compared when operating the atomizer at a fixed atomization-gas momentum flux.

In a collaboration with Science Applications International Corporation (SAIC), we have developed a new capability at NIST to determine the thermal properties and chemical reaction characteristics of solid and liquid multiphase (such as hazardous waste) substances through rapid and controlled heating with laser radiation. The Laser Driven Thermal Reactor (LDTR) is an off-line analytical device that will be used to complement the diagnostics capabilities of the NIST Spray Combustion Facility. The LDTR has been demonstrated by determining the integrated heat release rate for nitromethane. An improved understanding of the effects of heating rate and temperature on substance thermal and chemical behavior are important to efforts to enhance the energy efficiency of a process, and control the formation of chemical byproducts.

In FY 1997, we expect to direct our efforts toward using the spray combustion facility to provide benchmark experimental data for input/validation of multiphase combustion models. We are currently fostering ties with Dow Chemical, Babcock & Wilcox, and others, who are heavily involved in developing computational codes that they use to assist in improving the performance of thermal oxidation facilities. In collaboration with these companies we are currently defining a baseline case for which experimental data from the NIST combustion facility will be used for input/validation of these simulations.

Publications:

Aftel, R., Gupta, A.K., Cook, C., and Presser, C., "**Gas Property Effects on Droplet Atomization and**

Combustion in an 'Air-Assist' Atomizer," Proc. 26th International Symposium on Combustion (in press).

Cook, C.A., Charagundla, S.R., Presser, C., Dressler, J.L., and Gupta, A.K., "**Effect of Acoustic Atomization on Combustion Emissions**," in ASME Heat Transfer Division: Thermal Analysis in Waste Processing and Disposal 4, HTD-335,, Am. Soc. Mech. Eng., NY, 233-241 (1996).

18. Engineering Measurements for Hydrothermal Processes

***A. Lee**, W. Bowers, J. Horvath, W. Hurst, J. Maslar, D. Olson, O. Saulters, J. M. H. L. Sengers (838), W. Allmon (Babcock & Wilcox), H. Castillo (ACS SEED), C. Connon (Univ. of California, Irvine), J. Gallagher (Contractor), and M. Hodes (Massachusetts Institute of Technology)*

Objective: To develop *in-situ* measurement techniques that identify reaction and corrosion intermediates, and measure velocity and thermal profiles while withstanding the aggressive hydrothermal environment. In the longer term, to gather data for development evaluation of engineering design models.

Problem: Hydrothermal processes range from treatment of industrial wastes to power generation in boilers. Major challenges to the large scale implementation of these processes include inadequate destruction of particular compounds, vessel corrosion, salt deposition and scaling, poor understanding of the near-critical heat and mass transfer, and lack of on-line process sensors and engineering models. To overcome these challenges, it is necessary to understand and develop engineering models for the chemical reactions, transport phenomena, and thermodynamics in this medium. However, data acquisition for these models have been limited by a lack of robust, *in-situ* measurement techniques that can withstand the oxidizing, high temperature, high pressure, and high temperature density gradient environment. Thus, we seek to understand and model these important hydrothermal processes through development and application of *in-situ* measurement techniques.

Approach: Each project in the program addresses a major challenge, e.g., corrosion, reactor operation, salt deposition, and transport phenomena. For understanding corrosion processes and validation of other reaction mechanisms, Raman spectroscopy is used to identify key species. For developing reactor operation models, we investigate different oxidants and their ramifications on operating conditions, with results being quantified via an *ex-situ* (effluent) analysis for aqueous pH, specific ions, and total carbon. For studying salt deposition and heat transfer processes, experiments in various near-critical fluids, flow regimes, and chemical compositions will employ various visualization and measurement (*e.g.*, video, Schlieren interferometry, laser Doppler velocimetry, thermocouples) techniques. Salt deposition experiments will also provide phase boundary data, which supports thermodynamic modeling efforts.

Results and Future Plans: During FY 1996, we established that 514 nm laser light intrusively affected the *in-situ* observation of corrosion pre-cursor in supercritical water via Raman spectroscopy. However, recent results suggest that longer wavelengths will be less intrusive. *In-situ* observation of corrosion intermediates are critical for corrosion mechanism determination since we previously established qualitative differences with *ex-situ* observations. These measurements were conducted in our re-designed, state-of-the-art Raman cell which features increased signal collection, an electrically-isolated sample mount, and window corrosion protection via a thin diamond cover slip. This work is allied with Babcock & Wilcox and the Electric Power Research Institute, where we have improved the design, functionality, and durability of a Raman optical probe for *in-situ* chemical detection in boiler crevices. This probe is currently being used in the first ever commercial test application for crevice corrosion detection.

Waste destruction experiments, to support model development in a hydrothermal oxidation system, have also been conducted. With partial support of the Naval Surface Warfare Center, we investigated the performance differences between hydrogen peroxide and oxygen, on acetic acid and ammonia. We established that to serve as a reliable oxygen source, hydrogen peroxide should be heated above 670 K for quantitative thermolysis to oxygen. Furthermore, for overall efficiency, undecomposed peroxide has been found to be more effective than oxygen from 720 - 970 K, shown by the 260% reduction in overall activation energy for ammonia and acetic acid oxidation. This work has helped resolve a long standing inconsistency in the literature regarding the apparent performance similarities and dissimilarities between the two oxidants, and suggests operational alternatives for hydrothermal oxidation systems.

In a collaboration with the Massachusetts Institute of Technology, a new, low-cost flow cell was used to identify the mechanism of salt nucleation (homogeneous/heterogeneous) near a heated cylinder in cross flow, and to help estimate transport coefficients in near critical sodium sulfate solutions. The results demonstrated that nucleation on a hot surface is the dominant mechanism, and free stream nucleation could not be induced despite artificial seeding of the bulk phase. The rate and cumulative mass of salt deposited agreed well with a numerical model which suggested that the Lewis number (thermal diffusivity to mass diffusivity ratio) is less than 8 at these conditions. This demonstrates that these near critical conditions are much closer to ambient pressure gas-phase combustion conditions than for liquid water, where the relative mass diffusivity is nearly 2200 times slower. Both the experimental and modeling work were integrated with a new thermodynamic model to generate an equation-of-state for estimating densities and expansion coefficients of near-critical aqueous sodium sulfate mixtures based on a modified Anderko-Pitzer approach.

We also made substantial progress in the construction of our supercritical CO_2 heat transfer facility. The system will initially operate at 10 Mpa and from 270 K - 420 K, with heating rates of 40 kW/m^2, flows of up to 1 kg/s, and with variable orientation to study buoyancy effects.

For FY 1997, we expect to obtain corrosion spinel define data for several metal alloys and solution compositions, identify surface-to-volume and orientation effects on hydrothermal reactions and incorporate them into a process model, study the deposition of binary salt systems, and acquire heat transfer measurements in turbulent supercritical CO_2.

III. Surface and Microanalysis Science Division (837)

Rance A. Velapoldi, Chief

A. Division Overview

The Surface and Microanalysis Science Division conducts research to: 1) determine the chemistry and physics of surfaces, interfaces, particles, and bulk materials, and their interactions with a broad spectrum of species including electrons, photons, ions, atoms, and molecules; 2) determine the chemical and isotopic composition, morphology, crystallography, and electronic structure at scales ranging from millimeters to nanometers; 3) determine the energetics, kinetics, mechanisms, and effects of processes occurring on solid surfaces as well as within materials and devices; 4) study the total chemical measurement process as well as source apportionment in atmospheric chemistry using chemometrics; and 5) develop and certify Standard Reference Materials and Standard Reference Data. Emphasis is placed on performing fundamental and applied measurement research, providing data and standards (including software), and developing theories that are needed for accurately measuring chemical composition and dynamic processes that occur on surfaces and in microstructures. This information establishes the relationships between chemical composition at high spatial resolution and the effects of manufacturing processes, device operations, or material properties. The information also provides the bases for advances in various technologies, such as chemical catalysis, advanced electronics, and materials science.

Division staff were productive in technical research and program planning. In program planning, we focused our efforts in six major programs; these as well as selected accomplishments are given below to provide a flavor of the research as well as the directions that each program is going. Additionally, three new programs were identified and one was funded for FY97 through the CSTL planning process.

<u>Nanoscale Chemical Characterization:</u> Our objectives are to push the measurement envelope and provide infrastructural tools for the measurement communities to determine the chemical composition of various samples, ultimately at very high spatial resolutions and very low concentrations. In a broader context, the measurements include the chemical characterization (elements, isotopes, and molecules) of materials at millimeter to nanometer and smaller spatial scales with major, minor, and trace chemical concentrations. In addition to determining qualitative and quantitative chemical composition, the measurements also include crystallography (species and molecules) and morphology (structure). Instrumental methods include electron, ion, photon, and x-ray probes. These measurement tools can then be applied across a broad range of problems in the private and public sectors such that the 'microspatial' relationships of chemical species can be correlated with specific macroscopic properties of materials or devices as well as to determine the effects of individual manufacturing steps on a system or product.

Selected Accomplishments:

- Established zeptogram (10^{-21}g) analysis capability in the characterization of material for the nanoscale superconductor and nanoscale particle synthesis projects
- Began Competence Program by designing and building versatile atomic force microscope (AFM) with unique features including interchangeable sources and detectors, and piezo microphonics for constant atomic force sensing
- Characterized beam scattering in "low vacuum" environmental scanning electron microscope by measuring destruction of self-assembled monolayers with secondary ion mass spectrometry
- Installed and tested field emission analytical electron microscope with 0.09 nm resolution
- Developed visual tools for presenting results from multivariate image analyses

<u>Particle Characterization and Standards</u>: Our objectives are to focus on the special case of individual particles or samples that contain "particles" and to provide measurement capabilities to 1) characterize individual particles, and 2) characterize a sample by the chemical composition of each particle summed into "particle populations", a "micro-to-macro" concept. In this concept, automated measurement methods are developed by optimizing sample and instrument measurement parameters and developing or extending software for data collection, visualization, and interpretation. These methods are then used for the identification of samples, or contaminants, or to analyze particle starting materials or products in advanced materials production as well as in the development of particle SRMs. We also apply these infrastructure measurement capabilities to characterize actinides and other materials of interest; develop standards, data, and measurement protocols appropriate for use in quality assurance by various laboratories; and transfer these measurement capabilities to the laboratories of DoD, DoE, and international organizations such as the International Atomic Energy Agency (IAEA). These latter efforts aid the U.S. in monitoring the Nuclear Test Ban Treaty, determining the spread of nuclear weapons, aids in deterring terrorism, and helps the IAEA carry out their mission of worldwide nuclear monitoring.

***Selected Accomplishments*:**

- Developed automated system for scanning electron microscope (SEM) analysis of particles including random sampling, imaging, x-ray spectrum collection, and element quantification
- Developed visual and computer methods for interpretation of large spectral databases
- Developed and tested the Rapid Integrated Nuclear Analysis measurement protocol and applied it to the DoE Domestic Test Exercise
- Developed automated secondary ion mass spectrometry (SIMS) analysis to provide actinide isotopic ratio measurements on thousands of particles within 8 hours; transferred measurements to DoD, DoE, and IAEA
- Determined measurement parameters and developed measurement protocols for certifying "particles-in-oil" SRM
- Characterized TiO_2 nanoparticles by electron microscopy and electron holography to provide new three dimensional capability
- Developed micro-x-ray diffraction methods to characterize particles and thin films

Electronic and Advanced Materials Characterization: Objectives are to apply measurement techniques for the chemical and morphological characterization of electronic devices and components (e.g., semiconductors, superconductors, opto-electronics) and advanced materials (e.g., coatings, ceramics); and to develop needed standards and data. Examples of projects include 3-dimensional characterization [Semiconductor Industry Association (SIA) roadmap], round robin measurements using profilometry to measure craters and implant chemical distributions, and determination of major, minor, and trace chemical compositions with high spatial resolutions for starting materials and finished products.

***Selected Accomplishments*:**

- Developed 3-dimensional SIMS technique to characterize electronic materials
- Studied transient enhanced diffusion in low energy boron-implanted silicon to define production parameters for electronic devices
- Participated in round robin determination of boron depth distribution in silicon based on NIST SRM which decreased variation among laboratories by a factor of 7
- Established surface roughness sensitivity measurements with scanning scattering microscope to be 0.5 nm to 1.2 nm
- Applied ASEM and compositional mapping to characterize starting particles of yttria-stabilized zirconia and finished thermal barrier coatings on turbine blades in jet engines

Chemical Measurement Process: Objectives are to investigate the Chemical Measurement Process and establish generic methods, standards, data, and definitions, as bases for overall quality assurance. Several aspects of these objectives are an important aid to industry in achieving ISO 9000 certification requirements as well as providing the basis for traceability among national and international standards bodies, industries, and countries. Examples include: calibrations, data analysis/interpretation, process design, model validation, estimates of uncertainties and bias, Standard Reference Data (SRD) and Standard Test Data (STD), Standard Reference Materials, and Documentary Standards (i.e., terminology and reference procedures).

***Selected Accomplishments*:**

- Published definitive IUPAC document on the evaluation of analytical methods and definition of detection and quantification limits
- Developed and tested prototype STD for x-ray photoelectron spectroscopy (XPS) in collaboration with industry

- Built new ozone Standard Reference Photometer for Physikalisch-Technische Bundesanstalt (PTB), Germany (19 in international network: 12 in U.S. and 7 worldwide)
- Improved accuracy for inner-shell ionization cross sections for Auger electron spectroscopy (AES) and XPS
- Produced and characterized CO_2 isotopic reference materials for the international environmental, medical, and forensic communities

Surface and Interface Chemistry: The program develops measurement tools for the characterization of, provides fundamental data on, and produces models for, the basic chemical events in surface and interface reactions. Laser and electron spectroscopies and mass spectrometry are applied to the study of advanced surface processes including photoreactions at surfaces, energetic radical reactions with surfaces, and the details of thin-film growth and surface structure formation. Optical probes are also used to study both solid/solid and liquid/solid interfaces.

***Selected Accomplishments*:**

- Performed definitive experiments for ultrafast desorption processes; results support *ab initio*/friction theory
- Published complete theory for novel electron transfer mechanisms and control in femtosecond surface reactions
- Characterized electronic structure of doped oxide substances
- Demonstrated that exoergic H- atom induced D-abstraction involves significant substrate participation
- Used non-linear optical probes to differentiate between aligned and twinned $CoSi_2$ - Si interfaces

Advanced Isotope Metrology: Objectives are to develop basic procedures and provide tools to measure isotope ratios of chemical species in processes such as combustion, atmospheric gas-particle interactions and transformations, and contaminant transport, all critical for atmospheric source apportionment. Included here are the general objectives for chemical species source apportionment: 1) apply isotopic and molecular chemical characterization techniques, especially using Advanced Isotopic Metrology, to measure atmospheric contaminants on local, regional, and global bases; 2) determine sources of the contaminants; 3) model results considering meteorology to develop optimum sampling locations and strategies; and 4) provide results to interested sectors so they can devise control strategies for pollutant or contaminant reduction. Various emissions that can be differentiated include gas and diesel vehicles, power production, fast food frying, biomass and wood burning, and biogenic sources as well as chemical species used in or contaminants produced by manufacturing processes. Other isotope measurement capabilities include dual isotope dating, authentication, actinide analyses, and individual pulse characterization in low-level counting.

Selected Accomplishments:

- Provided first direct evidence for long range transport of carbonaceous products from biomass combustion (Canada to Greenland)
- Provided first evidence of isotopic fractionation in combustion of fossil and biomass fuels
- Prepared isotopically homogeneous $^{13}CO_2$ standards for international traceability of measurements
- Participated in source apportionment study (fossil/biomass) for Denver which led to state-legislated study to provide information for pollution abatement strategies

New Programs: Molecular Scale Chemical Characterization of Organic and Biomolecules: The critical dimensions of emerging technologies have shrunk to the point that current spatially-resolved surface-microprobes cannot meet the projected chemical measurement needs of industry. The Division intends to address this challenge *via* the development of two categories of advanced, non-destructive surface probes. The first is to leverage the NSOM competence project into a robust program by the development of chemically resolved scanned probe microscopies that would include chenical force AFM, heterodyne scanning tunnelling microscopy (STM), and AFM-NMR techniques. The second is to develop capabilities in spatially-resolved AES and XPS. The molecular information from the scanned probes, and elemental and oxidation state information from the probes of electronic states, will provide complementary, non-destructive information on surfaces of technological interest.

Other areas for future consideration as new programs include **Chemical and Process Information for Surface Engineering** and **Source Apportionment of Important Chemical Species**.

Staff Recognition:

Honors our staff received this year include: J. Greg Gillen, the Department of Commerce Bronze Medal for "...fundamental research in measurements and standards and leadership role in developing novel applications of ...SIMS including imaging of patterned self assembled monolayer films used as sensors, isotopically labeled tracers in botanical materials, and the localization of chemicals in biological materials for health and personal care."; and Eric B. Steel, Shirley Turner, Jennifer R. Verkouteren, and Eric S. Windsor, the Allen V. Astin Measurement Science Award "...for extraordinary efforts in providing technical support in all aspects of the bulk and air-borne asbestos accreditation program to protect the public......especially schoolchildren." These awards were based on long-term efforts that involved our mission of accurate measurements, standards, and data, as well as the critical aspect in the latter award of interacting extensively with the public.

Interactions: Strong interactions by the staff with our constituents and effective technology transfer are shown by substantial technical outputs, participation on 8 editorial review boards, close interactions with national (e.g., ASTM, ANSI, MAS, EMSA, NFPA) and international (e.g., ISO, IUPAC, IAEA, VAMAS) organizations, and participation on established committees. In addition, staff participated on evaluation and consulting panels for DoE, DoD, NSF, EPA, NRC, USGS, WHOI, and industrial groups. Finally, we have organized, co-organized, or participated in several international and national conferences.

B. Selected Technical Reports

1. *Improved Accuracy of Inner-Shell Ionization Cross Sections*

C.J. Powell

Objective: To provide cross sections with improved accuracy for the ionization of inner-shell electrons by electron impact.

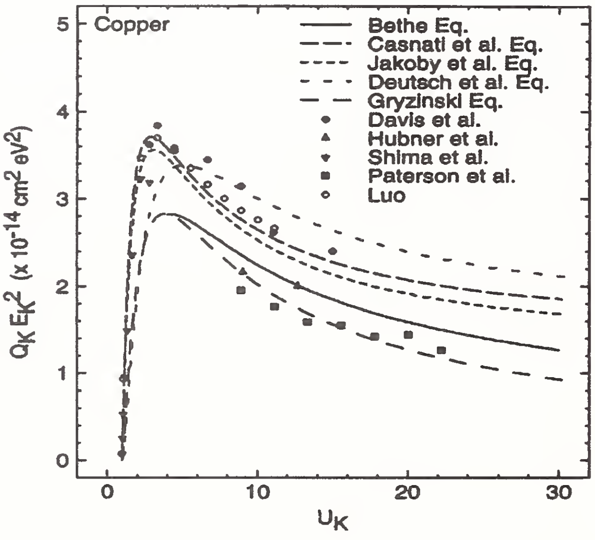
Problem: Cross sections for the removal of atomic inner-shell electrons by electron impact are needed for surface analysis by Auger-electron spectroscopy (AES), materials analysis by electron-probe microanalysis (EPMA), thin-film analysis by electron energy-loss spectroscopy, and for other applications in atomic physics, plasma physics, and radiation physics. For example, these cross sections are utilized in Monte Carlo simulations of electron transport in AES and EPMA to derive needed correction factors and to account for diminished or enhanced AES and EPMA signals from heterogeneous specimens.

Approach: A comparison has been made of measured and calculated K-shell ionization cross sections for eleven elements and of L-shell cross sections for two elements. These measured cross sections have been compared with theoretical values and with results from predictive formulae in common use to show the extent of agreement in the data from different sources and the trends with atomic number Z and electron energy E. The Figure shows an example of one of these comparisons with K-shell cross-section data for copper. It is convenient to plot the product of the ionization cross section Q_K and the square of the K-shell binding energy against the overvoltage U_K (ratio of E to the K-shell binding energy) since such plots are expected to vary slowly with Z. The solid symbols show experimental measurements, the open symbols are theoretical values, and the lines show evaluations from five formulae.

Results and Future Plans: The cross-section comparisons enabled trends and discordant data to be identified. For example, the K-shell cross sections for copper and other elements measured by Paterson *et al.* are lower than those from other laboratories, and appear to have a systematic error. For some elements, the high degree of consistency in measurements from different laboratories indicated that the relative standard uncertainty of measurement was less than about 10%, while for other elements the lack of consistency indicated that the relative standard uncertainty was 30% to 50%.

The Bethe equation, derived from a simple theory of atomic ionization, is useful for describing K-shell ionization cross sections but only if $U_K > 4$; this equation has two parameters which could be adjusted to improve agreement with experimental data. The more detailed theory of Luo provides generally good agreement with measured K-shell cross sections for many elements. An equation derived by Gryzinski from classical theory is widely used because of its simplicity; nevertheless, this equation gives cross sections that agree only roughly with measured values.

Several empirical equations have been proposed for the prediction of K-shell cross sections. The equation of Casnati *et al.*was found to be superior to those of Jakoby *et al.* And Deutsch *et al.*, and is recommended for use in future Monte Carlo simulations of AES and EPMA measurements.



Publications

Powell, C.J., **"Cross Sections for Inner-Shell Ionization by Electron Impact,"** Rev. Mod. Phys. (submitted).

2. Analytical Energy Dispersive X-ray Spectrometry with 10 eV Resolution by Microcalorimetry

*J.M. Martinis, D.A. Wollman, G.C. Hilton, K.D. Irwin, (EEEL, Boulder), and **D.E. Newbury** (CSTL, Gaithersburg)*

Objective: To revolutionize analytical X-ray spectrometry through the development of an energy dispersive X-ray spectrometer having the spectral resolution of a conventional wavelength dispersive X-ay spectrometer, i.e., approximately 10 eV.

Problem: Analytical X-ray spectrometry with either electron or X-ray primary excitation is performed with semiconductor energy dispersive X-ray spectrometry, which typically has a resolution measured at MnKα of 140 eV, and/or wavelength dispersive (i.e., crystal diffraction) spectrometry, which has a resolution of approximately 10 eV. EDS has the advantage of being capable of viewing the entire analytical X-ray spectrum from 100 eV to 30 keV (silicon detector) and to 100 keV (germanium detector). However, the poor resolution results in many peak interference situations that can severely degrade both qualitative and quantitative analytical accuracy as well as the limit of detection. Wavelength dispersive spectrometry has adequate spectral resolution to separate most interfering peaks and to provide high spectral peak-to-background ratios that are critical for trace element detection. It only views a narrow energy window (10 eV) and must be mechanically scanned to view the whole spectrum, limited to about 12 keV due to geometric constraints and the available crystal diffractors. This situation has now persisted for approximately 30 years.

Approach: A remarkable leap forward in X-ray spectrometry has been achieved by the EEEL Cryoelectronics Group by making use of microcalorimetry. A practical energy dispersive X-ray spectrometer with 10 eV resolution has been realized on a commercial scanning electron microscope platform. An X-ray photon is absorbed in a normal metal pad raising its temperature. The temperature rise, ΔT, is related to the ratio of the photon energy deposited, ΔE, and the heat capacity, C. The time duration of the temperature excursion is given by ratio of the heat capacity and thermal conductance. Ideally, we wish to have the largest possible temperature rise, to maximize the accuracy of the pulse measurement, and the shortest pulse time, to maximize the counting rate. This condition is best achieved at very low temperature, ~100 mK. The temperature pulse is measured with a "transition edge sensor" (TES) which consists of aluminum held at its superconducting transition within a heating feedback loop. Heat is conducted from the photon absorber to the low temperature heat sink through the TES. The excess energy of the photon is measured as the drop in the applied Joule heating (V^2/R) necessary to maintain the equilibrium of the TES. The reduction in current in the heating circuit due to an absorbed photon is detected and amplified through a superconducting quantum interference device.

Results and Future Plans: The electron-excited X-ray spectrum of barium titanate, which has a severe interference between the Ba-L family and the Ti K-family, is shown in Figure 1 for conventional EDS and the microcalorimeter. The resolution of the microcalorimeter is adequate to separate the interfering peaks for qualitative identification and quantitative intensity measurement. In the future we plan to field a microcalorimeter spectrometer in the Microanalysis Research Group in CSTL, Gaithersburg, to fully investigate its analytical uses, as well as opportunities to make fundamental physical measurements, such as the relative weights-of-lines within X-ray families.

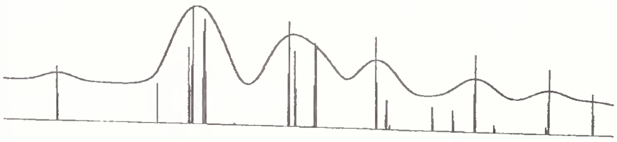


Figure 1. $BaTiO_3$ spectra: Lines = microcalorimeter; broad peaks = conventional 140 eV EDS; log counts vs energy.

3. *Discriminating Anthropogenic from Natural Sources of Individual Organic Compounds in the Environment*

L.A. Currie, T.I. Eglinton (Woods Hole Oceanographic Institution [WHOI]), *B.A. Benner, Jr.* (839), and *A. Pearson* (WHOI)

Objective: To extend atmospheric ^{14}C metrology to the molecular level through gas chromatography and accelerator mass spectrometry (GC/AMS); and to use this new methodology to quantify fossil and biomass sources of mutagenic, trace organic species (polycyclic aromatic hydrocarbons [PAH]).

Problem: Because of the serious health and climatic effects of chemicals released into the atmosphere, it is urgent to develop accurate methods for identifying and quantifying their sources. For carbonaceous emissions, the single most reliable fossil-biomass source discriminator is ^{14}C. Although there is little difficulty in measuring ^{14}C in major chemical fractions of carbonaceous aerosol, a problem arises if we wish to assign sources of individual, trace organic compounds, like the PAH, which pose a threat to human health. Results of prior research show that combustion aerosol is isotopically heterogeneous; therefore, fossil-biomass apportionment of individual compounds can only be achieved by isolating these compounds in sufficient quantity for direct ^{14}C measurement by AMS. The challenge was to demonstrate the feasibility of determining ^{14}C in individual PAH at the microgram per gram level.

Approach: This work was made possible through: 1) recent developments in AMS that allow the determination of ^{14}C in as little as ca. 100 micrograms of organic material; 2) the availability of a large quantity of a well-characterized "urban dust" Standard Reference Material (SRM 1649); and 3) the utilization of a unique, automated preparative capillary gas chromatography (PCGC) system at WHOI. A representative portion (ca. 21 g) of the SRM was processed at NIST by semi-preparative liquid chromatography to isolate a purified PAH fraction. Next, more than 100 automated injections of the PAH fraction were made using the PCGC system, to recover individual fractions containing from about 40 µg to 120 µg of the selected PAHs. These pure molecular fractions were then converted to carbon targets for ^{14}C AMS. A second material, SRM 1597 (PAHs from coal tar), was similarly processed to provide a ^{14}C-free control material.

Results and Future Plans: This experiment represents the first time that trace organic compounds have been individually radiocarbon "dated" in atmospheric aerosol. As anticipated, the *Urban Dust/Organics SRM 1649* shows diverse fossil/biomass source contributions to different classes of compounds, ranging from essentially all fossil (98%) for the aliphatic fraction, to 70% fossil for the polar fraction. The PAH dated, and the PAH fraction as a whole, contained about 90% fossil carbon. The *Complex Mixture of Polycyclic Aromatic Hydrocarbons SRM 1597* gave results for six individual PAH that were indistinguishable from pure, fossil carbon. Based on the dispersion of the six results, and the masses of the isolated compounds, we were able to estimate the standard deviation of the overall contemporary ("living") carbon blank to be 0.41 µg. This is *very* important, for it means that we have the possibility of applying this new methodology to practical environmental sampling studies. This research had two other important outcomes. First, it paved the way for certifying compound-specific carbon isotopic composition of environmental reference materials. Second, by direct ^{14}C assay, it gave support to the conclusion of a multivariate statistical model, that the PAH, benzo[*ghi*]perylene, can serve as a fossil fuel combustion tracer. This is important for future atmospheric research, considering the rapid disappearance of the conventional motor vehicle tracer, Pb.

Publication:

Currie, L., Eglinton, T., Benner, Jr., B., and Pearson, A., "**Radiocarbon 'Dating' of Individual Chemical Compounds in Atmospheric Aerosol: first results comparing direct isotopic and multivariate statistical apportionment of specific polycyclic aromatic hydrocarbons**," Nucl. Instrum. Meth., Proc. 7th Intern. AMS Conf., 1996 (in press).

4. *Pilot Study for Developing Standard Test Data: X-ray Photoelectron Spectroscopy*

J.M. Conny, L.A. Currie, and C.J. Powell

Objective: To develop simulated spectra for testing data analysis procedures and software in x-ray photoelectron spectroscopy (XPS) and use results as a general guide for STD development.

Problem: Standard test data (STD) are simulations of analytical instrument responses that help determine the veracity of procedures used in data analysis. STD are related to Standard Reference Materials (SRMs) in that both are used to assure the quality of the chemical measurement process, which consists of two domains: the sample preparation/measurement domain and the data evaluation domain. While SRMs are used to assess the complete chemical measurement process, STD are used solely for the data evaluation domain. The current focus is on STD for validating procedures for locating and determining the intensities (areas) of overlapping peaks in XPS, and for validating estimates of uncertainties in peak parameters.

Approach: The simulations in this study were based on measured carbon 1s spectra of polymers with a single C1s peak. Each measured C1s peak was smoothed and then fitted with a spline function, i.e., a series of polynomials of degree n joined together so that the derivative of order $n-1$ is continuous over the entire interval. The spectra were constructed, in most cases, by adding two spline-modeled C1s spectra to simulate a spectrum of overlapping peaks that might be measured from a specimen consisting of two polymers. Different doublet spectra were used to represent sample specimens exhibiting varying degrees of peak overlap, varying relative intensities of the two peaks, and varying levels of random (Poisson) noise (i.e., absolute intensities) for the combined spectra. The factorial-based design had three levels for peak overlap: a) no shoulder present, b) shoulder present, c) valley present. The design had three levels for relative intensities of the peaks: a)peak 1-high/peak 2-low, b) peak 1-low-/peak 2-high, c) peaks 1 and 2 approximately equal. The design also had two levels of Poisson rms noise: the higher noise level (i.e., lower absolute intensity) was twice the lower noise level (i.e., higher absolute intensity). For each spectrum in the design, multiple spectra were created by replicating the noise to simulate multiple measured spectra of the same specimen. In addition to doublet spectra of overlapping C1s peaks, spectra were simulated for single C1s peaks at the two levels of Poisson noise to provide the "null case" in the factorial design. Analysts who participated in this pilot study were asked to use their data analysis procedures to estimate peak binding energies and peak intensities and to estimate the uncertainties of these peak parameters.

Results and Future Plans: The study showed that STD are useful for validating estimates of peak parameter uncertainties when different spectral conditions exists, such as: 1) presence of a doublet *vs* a singlet, 2) doublets with varying degrees of peak overlap, and 3) doublets with varying intensities of component peaks. Results showed that 15-19% of doublets were identified incorrectly as singlets, while 30-32% of singlets were identified incorrectly as doublets. The rms deviation in *peak binding energies* from the true values was around 0.15 eV for peaks that were identified correctly as doublets. For singlets identified correctly, the rms deviation in peak binding energies from the true values was 0.13 eV for the low-noise spectra and 0.25 eV for high-noise spectra. When participants were asked to estimate their uncertainties in peak binding energies, estimates were 0.01 eV to 0.15 eV, substantially lower than the rms deviations overall for the group. The relative rms deviations in *peak intensities* from true values were 60% to 73% and 12% to 45% for doublets and singlets identified correctly, respectively. Surprisingly, in both cases, peak intensity uncertainties were higher for low-noise spectra than for high-noise spectra. Ongoing analyses of results from this study will help determine what 1) noise levels, 2) amounts of overlap, and 3) intensities of doublet peaks are appropriate for STD in XPS applications.

5. *Isotopic Gas Reference Materials for Global Measurements*

R.M. Verkouteren

Objective: To provide internationally recognized CO_2 Reference Materials (RMs) for the environmental, medical, and forensic communities that require well-characterized RMs covering the natural range of isotopic carbon and oxygen abundances.

Problem: Nearly all measurements of carbon and oxygen stable isotopic compositions in a variety of samples are performed through isotope ratio measurements of carbon dioxide derived from the sample, and standardized with measurements of reference CO_2. In recent years, improvements in automated instrumentation have allowed ^{13}C and ^{18}O abundances to be measured with relative variabilities (s, standard deviation) of about 0.001% and 0.002%, respectively, which enables the resolution of regional and temporal isotopic changes in environmental samples, and permits the use of natural (and safe) levels of stable isotopes in medical and industrial tracer studies. The combined standard uncertainty of any measurement, however, is dependent mainly on the uncertainty in the assigned composition of the reference CO_2. These uncertainties are typically an order of magnitude or more greater than measurement imprecisions since the laboratory reference CO_2 must be standardized through comparison with existing carbonate RMs. Standard CO_2 can be generated from these carbonate RMs only in small quantities and through a difficult chemical process that requires corrosive agents and precise temperature control, with isotopic reproducibility highly dependent upon the experience of the technician. The provision of carbon dioxide RMs for the standardization of laboratory reference gases should therefore improve not only the isotopic measurement capabilities of individual laboratories, but also improve the reproducibility of measurements made through international programs, necessary to harmonize standardization and to facilitate the interpretation of interlaboratory measurements. Carbon dioxide isotopically fractionates upon expansion, and isotopically exchanges with water contact and, to various extents, through contact with the oxygen-containing surfaces of glass and oxide films. Because of these problems, prior attempts to produce gaseous isotopic reference materials by filling vessels with CO_2 usually resulted in sample-to-sample relative isotopic heterogeneities (s) greater than 0.005% and 0.04%, respectively, for ^{13}C and ^{18}O abundances.

Approach: We designed and built a gas recirculating manifold containing a large capacity metal bellows (20 L) and a bank of seven parallel and replaceable borosilicate glass tubes. Cylinders containing three isotopically-distinct CO_2 gases were procured and treated in turn by identical methods. To allow isotopic exchange and equilibration, each gas was recirculated in the system for at least two weeks, then the RM units were generated by torch-sealing the CO_2 in each of the seven glass tubes into four equal-length sections. After the bank of glass tubes was used, the process was repeated with replacement tubes, eventually resulting in a batch of over 1000 RM units for each gas. Afterwards, each batch was heated to 70 °C for 24 hours to accelerate potential isotopic changes in the CO_2. Each unit was uniquely labeled while on the manifold and minor variations in production were documented, allowing the selection of units for identification of possible nuisance factors. Unique numerical labeling also allowed uniform sampling design of tube positions, lots, and chronology for representative measurement of isotopic reproducibility and analysis of variance. Isotopic measurements were performed at NIST and at the U.S. Geological Survey in Reston, VA.

Results and Future Plans: No nuisance factors were significant. Analysis of variance indicated that sample-to-sample isotopic variability between RM units was obscured by measurement imprecision. We estimated that the real isotopic variability of ^{13}C and ^{18}O abundance was less than 0.0007% and 0.0026% (s), respectively, for each batch of the RMs. At present, thirteen expert international laboratories are performing the difficult standardized measurements on these and other RMs, in order that compositional values and uncertainties may be assigned. We anticipate that the RMs, designated RMs 8562-8564, will be available through the SRMP early in 1997 with documentation complying with ISO Guide 31. We will monitor each batch for temporal isotopic changes, and are considering the use of the WWW for the dissemination of current information concerning these reference materials.

6. ^{14}C Measurements to Quantify Biogenically Produced Atmospheric Volatile Organic Compounds that Contribute to Ozone Formation

G.A. Klouda, G.C. Rhoderick (Div. 839), C.W. Lewis (US EPA), and R.A. Rasmussen (Oregon Graduate Institute)

Objective: To develop and evaluate a method to separate atmospheric volatile organic compounds (VOC) from whole air for ^{14}C measurement.

Problem: Atmospheric volatile organic compounds combine with nitrogen oxides (NO_x) and sunlight to produce molecules of ozone. The process, known as *photochemical smog*, produces ozone levels that often exceed the National Ambient Air Quality Standard (NAAQS) in many urban centers and rural areas throughout the United States. The NAAQS for ozone is 0.12 μLL^{-1} daily maximum over a 1-hour period. The problem in regulating ozone concentrations lies in knowing to what extent natural vegetative emissions contribute to ozone formation, *e.g., via* reactions involving biogenically-produced isoprene. An accurate measure of the radiocarbon (^{14}C) content of atmospheric VOC can give an accurate inventory of fossil and vegetative contributions and thus aid in more effective regulation of these ozone precursors.

Approach: The authors have used a combination of chemical and cryogenic means to isolate the VOC fraction from whole air for ^{14}C analysis. Samples are collected by pumping 0.1 m^3 of air into evacuated 32-L internally electropolished canisters. CO_2 is removed from the air by passing the air through a LiOH column and recollecting the air in another evacuated canister using liquid nitrogen. At NIST, the nearly CO_2-free air is processed through a vacuum manifold to trap the VOC fraction using liquid nitrogen, to combust the VOC to CO_2, and to reduce the CO_2 to graphite (as Fe-C solid solution) for ^{14}C measurement.

Results and Future Plans: The first ^{14}C-VOC results from two composited urban tropospheric air samples, collected during the summer (1992) in Atlanta, GA were published. The *upper limits* of the percentages of VOC originating from vegetative sources during the morning and evening hours in Atlanta were 9 and 17%, respectively; measurements reported at the 95% confidence level. Confidence intervals are relatively wide due to the process blank and its uncertainty. The results of these experiments, designed to 1) evaluate the entire VOC-^{14}C measurement process and 2) obtain reliable estimates of biogenic contributions to atmospheric VOC, emphasize how important controls are throughout the multiple-step chemical process to ensure quality data. Recent upgrades to the system are expected to lower the blank and additional measurements on samples collected in Houston, TX and Nashville, TN are planned.

This approach is being modified to extend the capability to 1 m^3 size samples for the separation of individual compounds, *e.g.*, atmospheric benzene; work currently supported by the American Petroleum Institute.

Publications:

Rasmussen, R.A., *et al.*, "**Removing CO_2 from Atmospheric Samples for Radiocarbon Measurements of Volatile Organic Compounds,**" *Environ. Sci. Technol.* 30, 4 (1996).

Klouda, G.A. *et al.*, "**Radiocarbon Measurements of Atmospheric Volatile Organic Compounds: Quantifying the Biogenic Contribution,**" Environ. Sci. Technol. 30, 4 (1996).

Klouda, G.A. and Connolly, M.V. "**Radiocarbon (^{14}C) Measurements to Quantify Sources of Atmospheric Carbon Monoxide in Urban Air.**" Atmos. Environ. 29, 22 (1995).

7. Refinements in Quantitative X-ray Microanalysis: Layered Specimens and Particles on Substrates

J.T. Armstrong

Objective: To develop and test algorithms permitting quantitative x-ray microanalysis of layered specimens and particles at a level of accuracy comparable to measurement for conventional thick, polished specimens.

Problem: Complicating the interpretation of analyses obtained by electron beam instruments is the fact that both the electron induced x-ray emission and its re-absorption can be greatly affected by the number and thickness of the various phases in a layered specimen or by the size and shape of an individual particle. The magnitudes of these effects change considerably with the energy of the x-ray and thus with the element that produced it. Compositions calculated from these intensities but not corrected for these effects often differ significantly with the sample's morphology, in extreme cases by as much as an order of magnitude. Hence, unless sample size, shape, and layering orientation are taken into account in correcting for electron scattering and x-ray absorption, one will not be able to determine particle compositions accurately, nor even determine if a group of films of different thicknesses and layering sequences or particles of different sizes and shapes have the same composition.

Approach: CITZAF is a package of correction programs, developed to provide state-of-the-art quantitative x-ray microanalysis of conventional, thick, polished, specimens. This program includes correction modules comprising all of the commonly used algorithms for quantitative electron microbeam x-ray analysis that are documented in the scientific literature, including the $\phi(\rho z)$ procedure developed for analysis of insulating materials. CIT-MCAR is a series of first-principles Monte Carlo trajectory calculation programs, adapted from previously published algorithms by Duncumb, Joy, Newbury, and Myklebust, that calculate emitted relative x-ray intensities from thick, polished specimens. The approach taken for this research was to: 1) extend the programs to include user-input sample boundary conditions (e.g., particle size and shape, multiple layer sequences of qualitative analysis); 2) adapt the electron scattering and x-ray absorption algorithms to the boundary conditions and thus develop correction algorithms for layered specimens and particles; and 3) test the resulting corrections on layered samples and particles of known compositions to determine the level of accuracy that could be attained.

Results and Future Plans: The programs have been modified to include correction for particles of a variety of geometric shapes and for a one-layered multiple-element thin specimen on a substrate. CITZAF has been incorporated as a working unit in the NIST-NIH Desktop Spectrum Analyzer (DTSA). In addition, programs by other investigators for analysis of layered specimens have been adapted and tested. The results of using these programs indicate that sources of systematic variability in multiple layered thin films can be reduced from the >100%-level to about 5% to 8% relative (with film thickness determination in agreement with other techniques such as Rutherford backscattering spectrometry). The results for individual particle analysis show a reduction in these effects from a level of as high as 1000% to a typical level of 5% to 10% relative (compared to 2% to 3% relative for conventional thick, polished specimens). Further testing is needed as well as implementation of multiple-layer specimens into the programs.

Publications:

Armstrong, J.T. **"ZAF, PAP, $\phi(\rho z)$, *a*-factor...: A comparison of the relative accuracies of the alphabet soup of correction factors for 'non-optimized' samples,"** Microscopy and Microanalysis '96, G.W. Bailey, N. Zaluzec and J. Michaels, Eds., Jones and Begell Publishing, New York (1996) 45-46.

8. A Study of an Yttria-Stabilized Zirconia Coating with Electron Microprobe Wavelength Dispersive Compositional Mapping

R.B. Marinenko, D.S. Bright, and E.B. Steel

Objective: To determine the compositional variation in Yttria-Stabilized Zirconia (YSZ) thermal spray coatings.

Problem: YSZ spray coatings are used as thermal barriers in many applications including critical components in commercial and military jet engines. The effectiveness and reliability of the thermal insulation provided by the coating depends on the chemical and physical properties of the YSZ. Chemical analysis of the coatings may help predict their performance. For example, the yttria content controls the cystal structure and the thermal expansion of the YSZ and low Yttria content is suspected of causing film failure.

Approach: Electron probe compositional mapping allows non-destructive analysis of the films with an analytical resolution of less than 1 μm and is being used to study the elemental distributions to help determine the variation of yttrium. Specimens generated at Sandia National Laboratories were analyzed. A series of x-ray maps were taken with the electron microprobe across cross-sections of the film, beginning from the interior iron matrix region, through the Ni bond coat and the YSZ film. The length of the region covered by the maps was approximately 0.75 mm. For each map, x-rays were analyzed for each element: Zr, Y, Fe, Ni, and Hf. The maps were 64 x 64 pixels, 50 μm along an edge, and measured for 2 s/pixel.

Results: A photomicrograph of the region traversed is in figure 1 where the area covered by each map can be seen from the contamination marks left on the specimen. The series of 15 quantified maps for Fe, Ni, Y and Zr are also in figure 1. The steel matrix and Ni bond coat are the bottom 4 maps with the YSZ coating starting in the fourth map from the bottom, continuing to the top. A quantitative matrix correction was perfomed on each pixel. Again, viewing from bottom to top, they show the quantitative abundance of each element. An inhomogeneous distribution of yttrium can be seen where areas of highest brightness, or highest yttrium mass fraction are approximately 8 wt.% and those areas of lowest mass fraction (darkest areas that are not voids) are less than 3 wt.%.

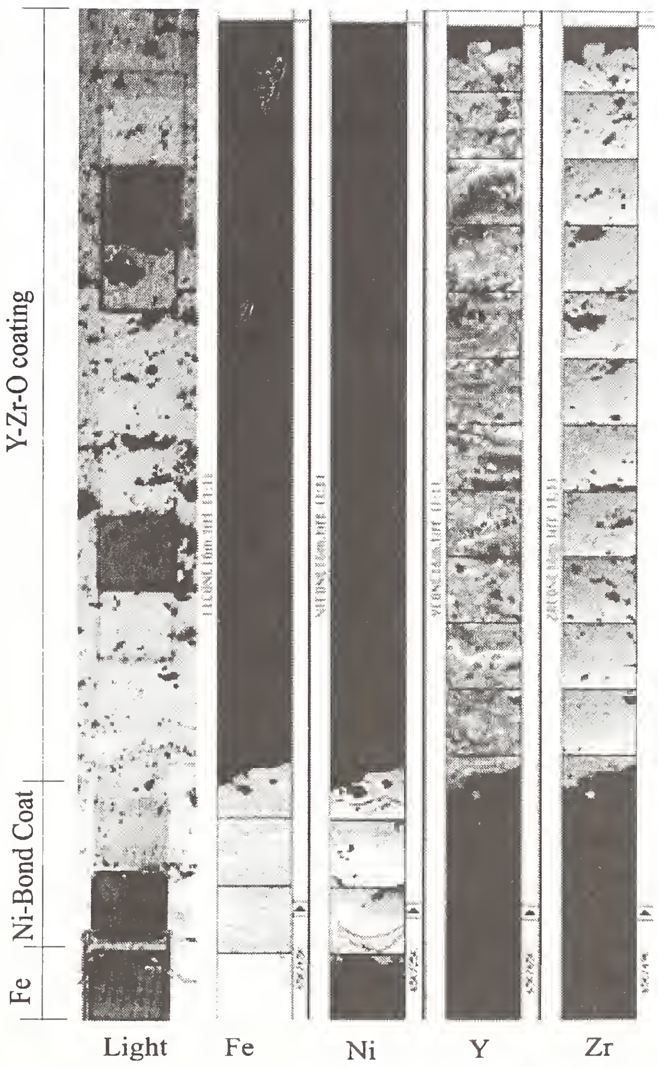


Figure 1. The first stripe is a photomicrograph of contamination rasters left from sequential WDS maps across the specimen surface of YSZ coated steel. Each of the next stripes is a fully quantitative chemical compositonal map for one element.

9. *Measuring Electron Beam Profiles in the Environmental Scanning Electron Microscope*

S.A. Wight and J.G. Gillen

Objective: To determine the scattering of the primary electron beam in the presence of a gas in the Environmental Scanning Electron Microscope (ESEM).

Problem: Danilatos reported on the presence of primary electrons scattered by the gas atoms in the elevated pressures of the ESEM. These scattered electrons effectively broaden the beam to include a "skirt" around the desired analytical area which limits the signal-to-noise of the electron imaging and the accuracy of qualitative and quantitative X-ray microanalysis.

Approach: A self-assembled decanethiol monolayer (SAM) is damaged by primary and scattered beam electrons. The damaged portion of the monolayer is exchanged with another thiol-containing molecule by immersion in solution. The resulting film is imaged using a secondary ion mass spectrometer. Three dimensional reconstruction of the data yields a representation of scattered electrons in the gaseous environment of the ESEM. The sensitivity of self-assembled decanethiol monolayers to electron bombardment in a high vacuum scanning electron microscope was previously demonstrated (Gillen, et al., 1994).

A spot beam was used in the ESEM exposure of the SAM that was intended to determine the fixed beam skirt under typical X-ray analysis and imaging conditions. The ESEM instrument conditions were: 20 kV, long working distance environmental secondary electron detector, 22 mm working distance, 50% condenser (3 nA current), no projection aperture, 300 second exposure, and water vapor pressure of 266 Pa (2 Torr).

After exposure to the beam, the damaged decanethiols were exchanged with fluoromercaptan by immersing sample in a 1 mmol $\cdot L^{-1}$ solution of fluoromercaptan in ethanol for one minute. The substrate was rinsed in ethanol and dried with nitrogen. Characterization of the resulting SAM was done by SIMS on a magnetic sector Cameca IMS-4F operating as an ion microprobe with a 0.2 μm beam diameter. The ion beam was rastered over the specimen and the secondary ions were collected to form an image.

Results and future plans: Electron dose to the SAM from primary beam scattering in the ESEM is represented as a three-dimensional plot in figure 1, which is the oxygen image from the 266 Pa (2 Torr) exposure. The white area is the highest intensity and corresponds to the primary electron beam striking the sample without scattering. The remainder of the raised area in the image corresponds to electrons scattered out of the beam.

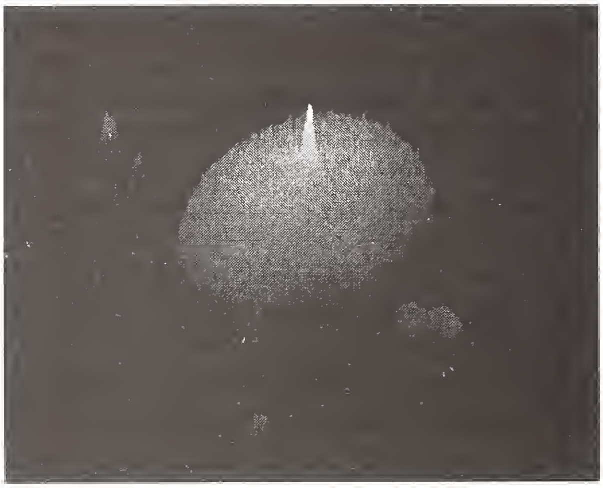


Figure 1. 3-D reconstruction of SIMS data for 266 Pa spot beam exposure. Image width is 300 micrometers.

Publications:

Gillen, G., Wight, S., Bennett, J., Tarlov, M.J.: **Patterning of self-assembled decanethiol monolayers on silver by microfocus ion and electron beam bombardment**. *Appl. Phys. Lett.* 65 (5) 534-536 (1994).

10. *Visual Tools for Multivariate Image Analysis: Rotatable and Projectable 3-D Scatter Diagrams Used with Principal Component Analysis*

D.S. Bright

Objective: To visualize trends and clusters in compositional image data.

Problem: Compositional Mapping often produces a number of registered images, each image representing the concentration of one element or chemical constituent. These images, viewed as a series of gray level images, give a general idea of chemical distributions in the sample, but figuring phase relationships using more than just a few images can be confusing. The series of maps in figure 1 is an example: Concentrations (intensities) should fall into distinct groupings according to the phases present in the material. Scatter diagrams of two or three of the images can help identify the phases in the material, especially when these images are chosen carefully. Figure 2 is a scatter diagram for three of the maps.

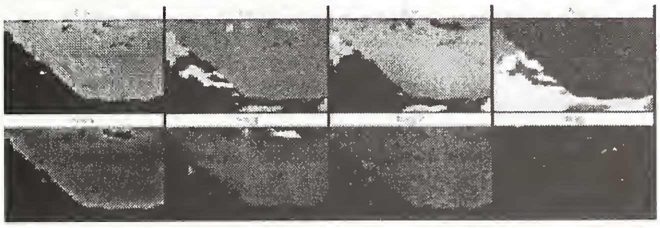


Figure 1. Compositional map of garnet

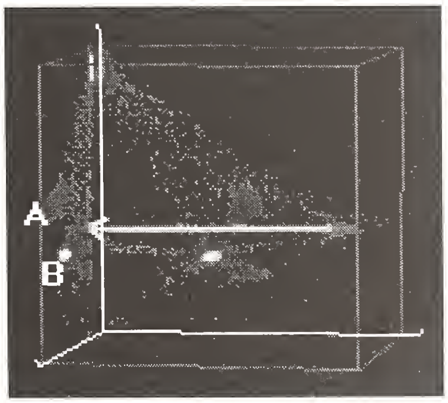


Figure 2. Composition scatter diagram

A three dimensional plot is often more useful than a two dimensional one, but it has the disadvantage of showing only a two dimensional projection of unfamiliar three dimensional shapes. For example, how far in and out of the plane of the paper is cluster A in relation to clusters B and C? This indicates the relative concentrations of calcium in each cluster.

Approach: Figure 3 shows a tool developed for this situation is the rotatable/projectable cube. It can be rotated with the mouse to view the diagram from another direction, and it shows faces (here the left one) onto which the 3D diagram can be projected. The faces are selected with a 'mouse click'. The diagram, along with the projection, is shown below in figure 4, and visualizes the relationship of the three clusters relative to calcium content: A>B>C.

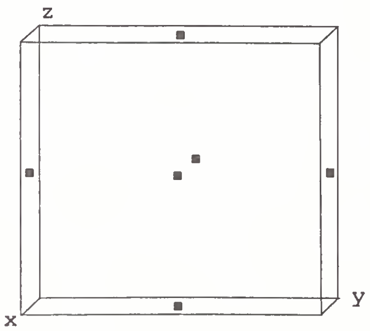


Figure 3. Projection cube

When many maps are in a data set, principal component analysis (PCA) is often used: a tool has been developed to convert a set of images into the corresponding set of PC images, which can be examined by scatter diagrams just as shown here. The advantage of PCA is that the first three components often show all of the interesting clusters in the data, eliminating the trial and error searching for the best images.

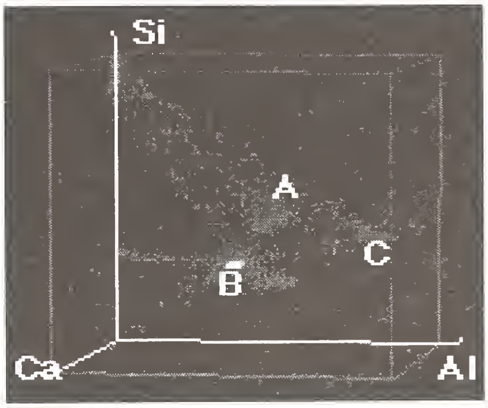


Figure 4. Replot of figure 2

11. *Automated Scanning Electron Microscopy (ASEM) Analysis of Particles: Data Reduction Using the Polyplot Package (P^3)*

J.A. Small and D.S. Bright

Objective: To develop a method to process data obtained from automated scanning electron microscopy analysis of particles and provide accurate population information.

Problem: ASEM analysis of particles results in the collection of several data sets including: particle X-ray spectra, particle images, particle morphology, and results from quantitative X-ray and multivariate analysis. To process the data, it is important for the analyst to sort and group the data within each data set, and to have the individual data sets linked through a common program.

Approach: We are developing a set of tools called the Polyplot Package (P^3) for processing the results from the ASEM analysis of particles. The P^3 tools enable the analyst to interact visually with the ASEM data sets to group particles and determine information on the particle population from the analysis of individual particles. The primary display for the P^3 is a polyplot of several hundred with one spectrum per particle. X-ray spectra. The X-ray peaks in the individual spectrum form patterns in the multi spectrum polyplot that can be used to discern rapidly elemental trends in the data set (see figure 1).

Results and Future Plans: Three groups of tools have been incorporated into the P^3. The first group of tools is used for sorting the spectra displayed in the waterfall plot. The entire data set can be sorted on total X-ray counts or on a specific characteristic X-ray peak intensity. After sorting, the spectra containing the peak of interest can be selected and placed in a unique particle group.

The second group includes tools for plotting up to three separate X-ray peaks and any of the morphological variables contained in the morphology data set. The plots include binary and ternary plots as well as three-dimensional plots. Individual points or groups of points can be selected from the plot and back-traced to the spectra in the polyplot enabling the analyst to select particle groups based on correlations between multiple variables.

The third group links the image data set and the polyplot. These tools are used to determine which particles have multiple analysis points. By selecting the multiple points on a given particle, the analyst can determine the particle homogeneity and group the spectra accordingly.

Two additional groups of tools are under development and will be incorporated into P^3 during FY 1997. These include a group of tools that allows the analyst to select particle elemental composition rather than peak intensity for sorting and plotting, and a group of tools that will link the polyplot to the results from a multivariate or cluster program.

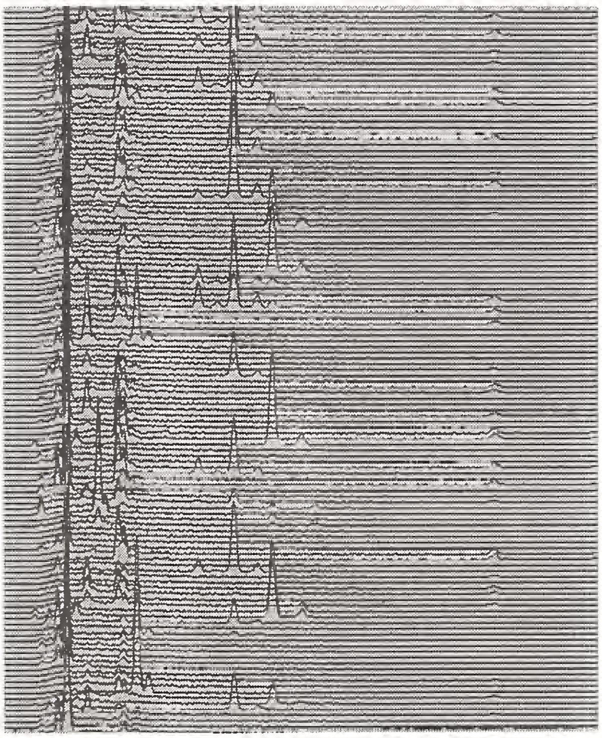


Figure 1. Polyplot showing X-ray spectra for individual particles

12. Characterization of TiO_2 Nanoparticles by Electron Microscopy and Electron Holography

S. Turner, J.E. Bonevich (MSEL), J.E. Maslar (836), M.I. Aquino (836), and M.R. Zachariah (836)

Objective: To characterize the morphology and microstructure of TiO_2 nanoparticles.

Problem: Nanophase TiO_2 (n-TiO_2) has been one of the most extensively studied of the nanophase ceramic oxide systems. n-TiO_2 has been found to differ from the larger-sized TiO_2 particles in a number of ways including improved catalytic activity and sinterability at lower temperatures. n-TiO_2 has been generated at NIST using a flame burner system. A goal of this work has been to characterize these nanoparticles and compare them to particles generated using other methods.

Approach: To generate n-TiO_2, titanium isopropoxide was aerosolized from solution and then oxidized in a flame fueled by oxygen and hydrogen. TiO_2 particles were collected for transmission electron microscopy on a carbon-coated grid at 2.5 cm and 5 cm above the base of a flame. Three temperatures were used ranging from 2000 K to 2500 K. X-ray diffraction analysis of particles collected on a cold finger showed formation of two TiO_2 polymorphs - rutile and anatase - in varying ratios. Individual particles from each sample were characterized using the bright-field, darkfield, and diffraction capabilities of the transmission electron microscope (TEM). The internal structure of some particles was characterized using electron holography, a new capability in our laboratory made possible by the recent installation of a TEM equipped with a field emission gun.

Results and Future Plans: Initial study of the particles shows that both the ratio of rutile to anatase and the morphology of the particles vary with flame temperature. At the lowest temperature, both rutile and anatase were generated. Particles occur as isolated grains, as bicrystals, and as chains of particles with planar grain boundaries. At the medium temperature, particles of both rutile and anatase are present with spherical or polygonal morphologies with the great majority occurring as isolated grains. At the highest temperature, the majority of particles are anatase with spherical morphology.

Two types of particles are particularly intriguing. Some of the larger particles generated at both low and medium temperatures show unusual central features that commonly are faceted. These central features were further characterized using electron holography. This technique allows for determination of the phase change of the electron beam as it traverses a particle. The phase change is directly related to the chemical composition and thickness of the material. A hologram of one of these particles is shown below. Comparison of the profile of the experimental phase image obtained from the hologram to a calculated phase image shows that the phase image is consistent with the presence of a void in the particle.

A second type of particle of interest is the spherical particles. Diffraction patterns obtained from the particles show that they are single crystals. Further studies are planned to reconcile this finding with the morphology of the particles. Use of techniques such as nanodiffraction and high-resolution TEM is planned.

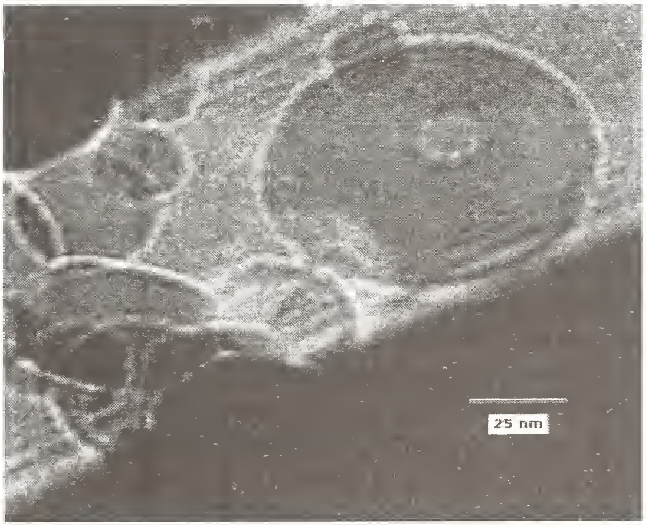


Figure 1. Hologram of n-TiO_2 particle

13. Femtosecond Laser-Induced Desorption of CO from Cu(100): Comparison of Theory and Experiment

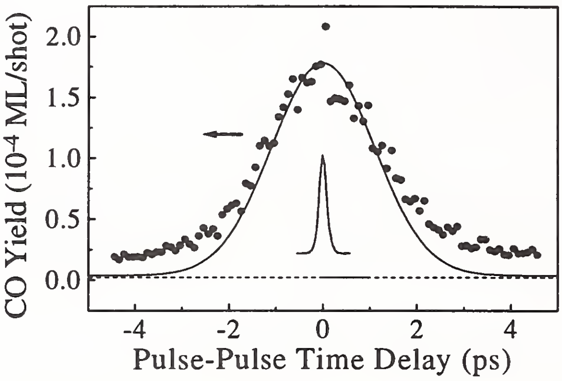
L.M. Struck, L.J. Richter, S.A. Buntin, R.R. Cavanagh, and J.C. Stephenson (844)

Objective: To test state-of-the-art theoretical predictions of femtosecond laser-induced desorption of CO from Cu(100).

Problem: The ability of femtosecond lasers to stimulate novel surface reactions has been of significant interest, as it offers the potential to modify surface layers without the limitations associated with normal heating processes. For small adsorbates on metal surfaces, a number of groups in academia and industry have reported novel desorption channels that were activated by femtosecond optical pulses. While there has been general agreement among different laboratories that the desorption process was prompt and that the desorption yield was highly non-linear in the absorbed laser fluence, there has been considerable debate regarding the mechanism involved.

Approach: We have undertaken state-resolved measurements of CO desorbed from 0.5 monolayer (ML) of CO on Cu(100) in an effort to better understand femtosecond laser-induced desorption. The CO/Cu system was chosen because its thermal properties have been well characterized and it was the focus of recent *ab initio* calculations of femtosecond laser-induced desorption. Pulses from a Ti:sapphire laser (400 nm, 160 fs) desorbed CO from a Cu surface held at 100 K. The desorbed CO was detected in a quantum-state-specific manner using resonance enhanced multiphoton ionization. The fluence dependence of the desorption yield, the pulse-pulse correlation of the desorption yield, and the quantum state populations (rotational, translational, and vibrational) were measured and provided a basis for comparison with predictions of both an *ab initio* theory and an empirical friction model.

Results and Future Plans: Over the fluence range 20 J/m^2 to 50 J/m^2, the CO yield was described by a power law with n=8±1. At a fluence of 46 J/m^2, the effective translational temperature was 215±15 K, the rotational temperature was 225±25 K, and the CO vibrational temperature was 1330±275 K. The empirical friction model predicts a yield which agrees with experiment, and a CO vibrational temperature at the time of maximum desorption yield of 1300 K. The predicted translational and rotational temperatures are both 400 K, in reasonable agreement with experiment. Shown in the figure is the CO desorption yield as a function of pulse/pulse time delay. The line through the correlation data is the prediction of the empirical model. The narrow peak is the autocorrelation trace for the 400 nm pulses.



The experimental results were also compared to recent full-dimensionality classical trajectory calculations. Both the empirical model and the trajectory calculations agree quite well with the experimental results, indicating that for CO/Cu(100) frictional model accounts well for the femtosecond desorption data.

A letter summarizing the highlights of this work has been published, and a detailed paper is being prepared that incorporates an extended discussion of these results and more experimental details.

Publication:

L.M. Struck, L.J. Richter, S.A. Buntin, R.R. Cavanagh and J.C. Stephenson, **Physical Review Letters <u>77</u>**, 4567 (1996).

14. *H Atom Abstraction Reactions from D/Si(100)*

S.A. Buntin

Objective: To characterize the operative mechanism for the H-atom induced abstraction of D adsorbed on a Si(100) surface.

Problem: Surface reactions are generally described by two mechanisms that represent disparate limits of energy accommodation between the reactants and the substrate. For the Langmuir-Hinshelwood mechanism, the reaction proceeds with the reactants fully accommodated with surface energy. In the other limit, a gas phase species can react directly with an adsorbate, with no energy transfer to the substrate. This Eley-Rideal (ER) mechanism, while suggested nearly fifty years ago, has been observed conclusively for exoergic atom abstraction reactions on metal surfaces only recently. While evidence for an ER process in the H-atom abstraction of adsorbates from semiconductor surfaces exists, conclusive dynamical studies are needed to validate this interpretation.

Approach: To probe the details of the reaction mechanism, the kinetic energy distribution of the product HD formed from the reaction of gas phase H atoms with D adsorbed on Si(100) is measured as a function of incident H atom kinetic energy. The D adlayer on Si(100) is prepared by exposing the clean surface held at 650 K to a flux of D atoms formed by thermal decomposition of D_2; under these conditions the monodeuteride surface species (i.e., a single D atom bound to each Si surface atom) is present. H atoms are produced by excimer laser photolysis of HI, which is admitted into the vacuum chamber with a pulsed valve. The energy of the incident H atoms is selected by varying the laser wavelength and polarization; incident energies of nominally 1.0 eV, 1.9 eV, and 3.3 eV are formed using 248 and 193 nm photolysis. The product HD kinetic energy distributions are determined from time-of-flight (TOF) spectra measured with a quadrupole mass spectrometer.

Results and Future Plans: The TOF spectra for incident H atoms having energies of 1.0 eV, 1.9 eV and 3.3 eV are indicative of HD products having nominally *identical* mean kinetic energies. While the data analysis is still in progress, it is apparent that the HD products are highly excited translationally, with mean kinetic energies greater than 1 eV. There are subtle differences in the TOF spectra, with the more energetic incident H atoms showing slightly broader distributions. Given the reaction exoergicity of about 1.0 eV, it is apparent for the 1.0 eV incident H-atoms that a significant fraction of the available energy (reaction exoergicity plus incident H-atom energy) appears in product translation. This is consistent with an ER process. The observation of the product mean kinetic energy being insensitive to incident H-atom energy is, however, not consistent with the naive expectation for an ER process. For an ER process, given that the available energy for the 3.3 eV incident H atoms is about twice that for 1.0 eV incident species, one would expect the product kinetic energy to be greater for the higher incident energy species. It is possible that the increase in the available energy channels into excitation of the product HD internal modes (i.e., vibration and rotation), which are not probed by these TOF measurements. Rationalizing the data based on this effect alone is problematic, however, since the observations require that *all* of the additional incident energy appear as HD internal excitation. A more likely scenario, based on this preliminary analysis, is that the substrate modes are involved in this reaction and provide an energy sink.

Initial conclusions from this work that the exoergic H-atom induced abstraction of D from Si does not occur in the ER limit, but involves significant substrate participation. Measurement of the internal excitation of the HD product would provide critical information necessary to unequivocally address the energy disposal and reaction dynamics for this system. This type of mechanistic characterization is imperative for the continued development of reliable, accurate computer-based modeling of surface reactions that occur in activated semiconductor process environments.

15. Probing Buried Interfaces with Non-linear Optical Probes: Si/CoSi$_2$

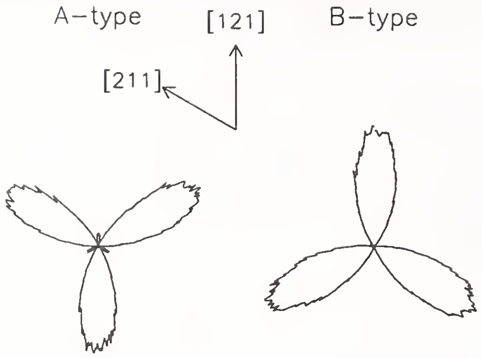
L.J. Richter, K.W. Kolasinski, and J.C. Stephenson (844)

Objective: To characterize non-linear optical techniques as probes of Schottky barrier geometric and electronic structures.

Problem: As semiconductor device design rules shrink, performance is increasingly dependent on interface quality. However, few diagnostics exist that can characterize buried interfaces; traditional charged particle probes can only access model interfaces between substrates and ultrathin films.

Approach: We have undertaken second harmonic generation (SHG) and sum frequency generation (SFG) studies of Schottky barriers formed between Si(111) and (100) oriented substrates and CoSi$_2$. SHG and SFG have the traditional advantages of optical probes: they are nondestructive and can be used *in-situ*/on line; additionally, as nonlinear techniques they have the added advantages of being uniquely interface sensitive (bulk second order mixing is forbidden in centrosymmetric media such as Si and CoSi$_2$) and having greater sensitivity to interface structure. The Si/CoSi$_2$ interface is ideal for studies of Schottky barriers as it is atomically abrupt and epitaxial and has been extensively characterized both experimentally and theoretically.

Results and Future Plans: Si /CoSi$_2$ interfaces were prepared by ion beam mesotaxy. The resulting samples consisted of nominally 20 nm of crystalline Si on top of 60 nm of CoSi$_2$ on a crystalline Si substrate. For (111) oriented substrates, two interface structures can be formed, aligned or A-type and twinned or B-type. The mesotaxy could be optimized to produce interfaces predominantly of each type. Shown in the Figure is a polar representation of the p-polarized SHG intensity at 532 nm, as a function of the angle between the plane of incidence and the indicated Si crystal axes for both A and B-type (111) interfaces. The 1064 nm fundamental was p-polarized and the angle-of-incidence was 45°. One can clearly identify the twinned interfaces by the 180° rotation of the SHG patterns.



Conventional linear optical techniques cannot make this distinction. The overall quality of the data establishes that non-linear optical techniques are well suited to the study of these buried interfaces.

Spectroscopic studies of all 3 interfaces: (111) A, (111) B and (100), have been performed *via* SFG between a fixed 1064 nm probe and a tunable, 1400 nm to 2200 nm probe. Previous SFG studies of disordered Au/GaAs Schottky barriers have observed resonant SFG response due to interface-localized defect states in the GaAs band gap. These states have been associated with Fermi-level pinning. Our studies of the well-ordered CoSi$_2$/Si interfaces did not observe localized states in the Si gap. For all 3 interfaces, the SFG response was observed to increase smoothly for tunable probe photon energies below the Schottky barrier height, possibly due to density-of-states effects at the metal/semiconductor interface. Theoretical models for the observed SFG response are being sought.

A manuscript discussing both the SHG and SFG studies is in preparation.

16. *Surface Femtochemistry with Fast Lasers and Slow Nanostructures*

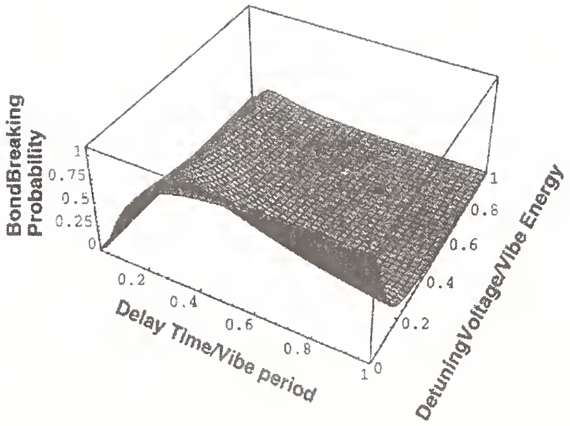
J.W. Gadzuk

Objective: To develop novel, non-thermal methods for controlled atomic-level manipulation and chemical processing at solid surfaces.

Problem: A promising realization of femtochemistry, based on inelastic resonant scattering of hot electrons by the reactants, achieves control of the intra-molecular dynamics required for select chemical processes, by controlling, in an appropriate way, the energy distribution of the hot-electron flux upon the surface. The major technical challenges are twofold. First, a useful theoretical model for the process must be developed which provides the causal relationships between a desired chemical output and the characteristics of the incident, hot- electron distribution. Secondly, lab systems capable of producing the required hot electron distributions must be realistically attainable.

Approach: The modeling and theory for two radically different methods of electron control have been developed. The first method uses an energetic broadband distribution of hot electrons, as realized in most laser-excited surface experiments. The second method is based on a novel application of a solid state nanostructure or tunnel junction, which produces a tuneable hot-electron flux internally incident upon the metal-vacuum surface where the controlled surface femtochemistry occurs *via* resonant scattering through an adsorbate negative-ion-state.

Results and Future Plans: A unified theory for both laser-excited and tunnel-junction surface femtochemistry has been developed and the initial presentations have been published. The theory provides analytic expressions for the chemical reaction probability (bond-breaking or desorption) given as an integration on energy of the device-dependent incident hot-electron flux over the resonant cross section for the reaction channel. The reaction probability is expressed as a function of the control parameters, namely the time-delay between the birth and death of the negative-ion state and the detuning voltage across the junction, as shown in the Figure, where the control parameters are specified relative to the vibrational characteristics of the bond which is broken. Indeed substantial control of the reaction probability is seen to be possible with realistic variations of the laboratory controls.



In FY 1997, the theory of tunnel junction femtochemistry will be further advanced to exploit the relevant solid state device potential. Other novel applications of this emerging technology, particularly as they may pertain to electrochemistry, sensors, and/or scanning tunneling microscopy, will be investigated.

Publications:

Gadzuk, J.W., **"Hot Electron Femtochemistry at Surfaces: Control in Desorption"**, in "Femtochemistry: Ultrafast Chemical and Physical Processes in Molecular Systems", ed. by M.Chergui (World Sci., Singapore, 1996), p.465.

Gadzuk, J.W., **"Resonance-Assisted Hot Electron Femtochemistry at Surfaces"**, Phys.Rev. Letters **76**, 4234 (1996).

17. *Measurement of Dead-time and Characterization of Ion Counting Systems for Mass Spectrometry*

A.J. Fahey

Objective: To develop a method to characterize electron multiplier-based pulse counting systems. Specific attention is paid to measurement of the counting system dead time. The systems are typically used to measure the abundance of ions passing through a mass spectrometer. The method uses measurements of isotopic ratios in a sample of known composition.

Problem: Accurate and precise measurements of isotopic ratios require a detailed knowledge of the behavior of the electron multiplier counting system. The dead time associated with pulse-counting must be known accurately and precisely, and be verified to be independent of count rate and isotopic pair chosen. Conventional measurements do not test this independence, and may underestimate the dead-time uncertainty.

Approach: The isotopic ratios of element X can be measured and potential mass interferences either excluded or corrected. If the measurements have been made properly, two systematic effects will be present in the data. These effects are readily observable when the data are plotted as delta-values, normalized to the most abundant isotope. If isotopes 2 and 3 are ~10 times less abundant than isotope 1, the dead-time effect at those masses is small. Isotope 1, assumed to be the normalizing isotope, has a delta-value of zero by definition. A line drawn through the delta-values of isotopes 2 and 3 has a non-zero slope due to mass fractionation. The extension of this line to the position of isotope 1 does not intersect at a delta-value of zero due to the dead-time effect. The dead time can be computed from the intersection of the line drawn between the delta-values of isotopes 2 and 3 at the position of isotope 1. The delta-values for isotope *i* is given by:

$$\Delta^i X = \left(\frac{{}^iX_{Meas} / {}^1X_{Meas}}{{}^iR_{Ref}} - 1 \right) \times 1000 \qquad 1$$

We can assign:

$${}^iR_{Ref} \approx {}^iX_{True} / {}^1X_{True} \qquad 2$$

If we designate the intersection of the line at isotope 1 as "δ", we can write

$$\delta = \Delta^2 X + (m_2 - m_1)\frac{(\Delta^2 X - \Delta^3 X)}{(m_3 - m_2)} \qquad 3$$

Where m_1, m_2, and m_3 are the masses of each of the isotopes, respectively. Substitute equations 1 and 2 into 3 to get:

$$\delta / 1000 = {}^1X_{True} / {}^1X_{Meas} - 1 \qquad 4$$

Since ${}^1X_{True} \approx {}^1X_{Meas}$, the dead time can be approximated by:

$$\delta / 1000 \approx {}^1X_{Meas} \times \tau \qquad 5$$

This procedure allows a determination of the dead time as a function of the count rate of the major isotope, and provides a method by which to investigate the of behavior of the counting system.

Results and Future Plans: The raw delta-values for Ti isotope measurements from a secondary ion mass spectrometer are shown in figure 1.

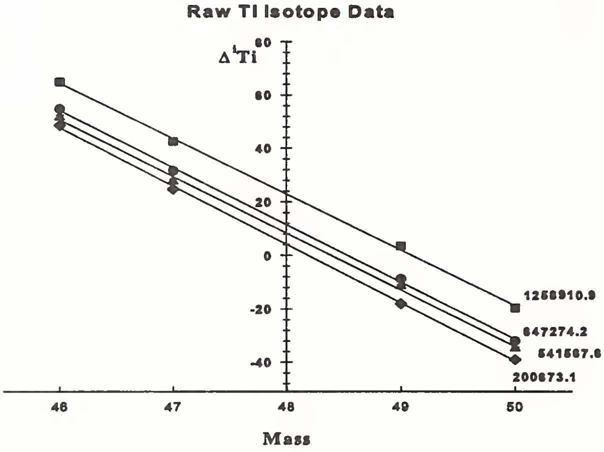


Figure 1. Ti isotope delta-values at various count rates

The dead time for the measurements in figure 1 was 17 with an expanded uncertainty of 1 ns and was independent of count rate. The intercepts of the lines vary due to the dead time. The linear dependence of the delta values due to mass fractionation can also be seen. These measurements on a system such as Ti with three or more isotopes allow the dead time and mass fractionation to be assessed independently, and provide a realistic estimate of the dead-time uncertainty.

18. *Comparison of Three Alpha Autoradiography Methods*

C.J. Zeissler

Objective: To determine the performance characteristics of three alpha autoradiography methods applied to environmental radioactivity characterization.

Problem: Bulk spectrometry or radiochemical methods often used to characterize environmental radioactivity fail to retain the individual characteristics of discrete microscopic radioactive particles. Alpha autoradiography, however, offers a means to characterize radioactive particles on an individual basis while they are still distributed in a bulk sample. Autoradiography can also be used for bulk radioactivity characterization. The performance characteristics of three alpha autoradiography methods are compared for these applications.

Approach: A sample consisting of dispersed $^{238}PuO_2$ particulate was used to perform autoradiography by the following three methods: 1) Nuclear track-etch (NTE), consisting of polyallyl diglycol carbonate, chemically etched and optically scanned; 2) Semiconductor array, consisting of live-time processing and display of alpha particle signals recorded in a charge-injection device detector (CID), and 3) Laser-scanned photostimulated luminescence (PSL), using a $BaFBr{:}Eu^{2+}$ phosphor. Parameters measured include spatial resolution, signal to noise ratio, active area, display rate, response linearity, detection sensitivity, dynamic range, and relative efficiency. Practical considerations were also determined.

Results and future plans: Optimal measurement parameters were determined for the systems: 2×10^{-4} Bq point sources can be located with a 48 hour exposure (NTE, CID); spatial resolution for point source location is 10 µm with an expanded uncertainty of 1 µm (NTE); detection surfaces can exceed 20 cm x 25 cm (PSL); and 2π quantum detection efficiencies are about 80% (NTE, CID). The alpha count response or total signal with respect to exposure time is linear over the range of activities tested (CID, PSL). Background signals are as low as 10^{-5} $\alpha/h/mm^2$ (NTE). Results can be accumulated live-time during the exposure (CID) or within 15 minutes following an exposure (PSL) without the need for chemical processing.

The main conclusions from this study are that no one method combines optimal performance for each capability, and one or a combination of the methods can provide useful radioactivity characterization of multiple discrete alpha-emitters. Work planned for FY 1997 includes further evaluation and characterization of the phosphor system, a collaborative comparison with an imaging spectrometer developed by other researchers, and the incorporation of analytical microscopy to determine chemical species of individual radioactive particles.

Publications:

Zeissler, C.J., "**Comparison of Semiconductor Pixel Array, Phosphor Plate, and Track-Etch Detectors for Alpha Autoradiography**", Proc. 4th International Conference on Position-Sensitive Detectors, Manchester, U.K., Sept. 9 - 13, 1996. Nuclear Instruments and Methods A (in press).

19. *Automated SIMS for Determining Isotopic Distributions in Nuclear Monitoring*

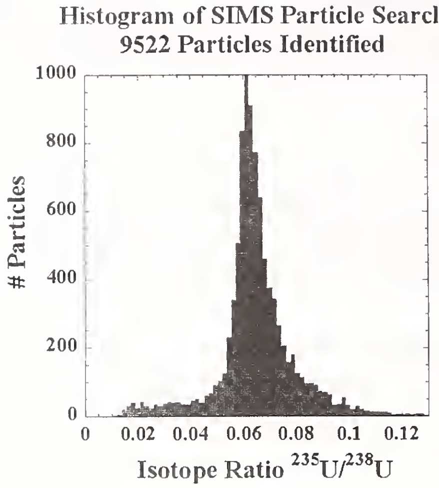
J.G. Gillen and D.S. Simons

Objective: To develop new analytical techniques for the automated analysis of isotopically perturbed particles to determine the isotopic composition of nuclear materials.

Problem: The International Atomic Energy Agency (IAEA) is tasked with monitoring world wide nuclear activities 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. As part of their safeguards program, the IAEA is routinely invited to conduct on-site inspections of civilian nuclear facilities to confirm inventories and verify the enrichment levels and compositions of nuclear fuels. During these inspections, samples of certain types of safeguarded materials are taken for later analysis by IAEA analytical laboratories. We are assisting the IAEA by developing new analytical methods for the characterization of some of these materials.

Approach: Because nuclear materials have distinctive isotopic signatures, it is possible to use mass spectrometry to detect and measure their isotopic compositions (and thus the enrichment level) on very small amounts of material. We are developing imaging secondary ion mass spectrometry (SIMS) as a tool for rapid screening of the isotopic composition of particulate matter collected by the IAEA during safeguards inspections. The advantage of this approach is that very little material is needed and sample throughput can be very high. Particles are collected at various locations within an inspected facility by wiping exposed surfaces (walls, floors, processing equipment) with a special cloth wipe. The collected particles are washed from the wipe, put into suspension using an appropriate liquid and dispersed onto the surface of a pyrolytic graphite disk for insertion into the secondary ion mass spectrometer. Analysis involves bombarding the sample with an energetic oxygen primary ion beam. This bombardment results in the emission of characteristic secondary ions which can be used to generate images for the isotopes of interest (typically the ^{235}U and ^{238}U isotopes of uranium). The field-of-view for the images is 150 µm in diameter with a spatial resolution of 1 µm. After image acquisition, custom image processing software is used to determine the isotopic composition and location of the nuclear particles contained in the analyzed area. After completion of an acquisition cycle (1 min to 2 min), the sample stage is translated under computer control to an adjacent area and the analysis is repeated.

Results and Future Plans: Using this method, the isotopic ratio of large numbers of particles can be determined in a few hours. Over a several week period in FY 1996, we measured the isotopic composition of well over 60,000 individual particles from IAEA test samples. The data from one typical analysis is shown below in a histogram display. In this example, 9522 uranium particles were identified and their ^{235}U/^{238}U ratios measured during eight hours of automated analysis. Our evaluation of this technology for IAEA applications will continue in FY 1997 and we are developing isotopic particulate standards for characterization of our experimental procedures.



20. Particle Analysis Using Micro X-Ray Diffraction Techniques

J.R. Verkouteren and E.B. Steel

Objective: To use micro X-ray diffraction (XRD) to analyze particles too small for conventional powder XRD and to perform spatially resolved X-ray diffraction.

Problem: One of the goals of our group is to perform thorough chemical and physical characterizations of individual particles. We have not been able to characterize the crystalline phase of individual particles that are too small to be analyzed by conventional XRD but too large to be analyzed by TEM without additional sample preparation.

Approach: To address this need, we are developing an expertise in micro X-ray diffraction, a technique that is commercially available but relatively uncommon. A microdiffractometer with a position-sensitive detector mounted on an 18 kW rotating anode X-ray generator was used. Four interchangeable beam collimators provide nominal beam diameters of 100 µm, 50 µm, 30 µm, and 10 µm. The sample is mounted on a goniometer that has three axes of rotation, allowing the sample to be oscillated to obtain a powder diffraction pattern. The position-sensitive detector simultaneously detects all diffracted rays over a 2θ angular range of 5° to 140° and the data are displayed using a multi-channel analyzer.

Results and future plans: Three different types of materials were investigated using micro X-ray diffraction, including two particulate samples and one thin film. In the case of the first particulate sample, the problem was to identify the crystalline phase. A number of micrometer-sized particles with the same chemistry had been isolated and mounted in collodion for analysis by conventional XRD. The sample was analyzed for 36 hours, and still the diffraction pattern was extremely weak and overwhelmed by a collodion background. One of the particles, a thin plate approximately 100 µm wide and less than 10 µm thick, was separated from the collodion and analyzed by micro X-ray diffraction and the phase was easily identified as orthorhombic uranium metal.

The second particulate sample is comprised of polycrystalline spheres of zirconium oxide that range in size from micrometers to millimeters. XRD analysis of the bulk material shows the presence of many forms of ZrO_2, including the monoclinic, tetragonal, orthorhombic, and cubic forms. The question was whether each individual sphere was also multiphase, or whether differences existed among the spheres, particularly at the smaller size range. We have analyzed 11 spheres and have found differences in the phase distribution, even for the larger particles. The smallest particle we have analyzed to date is approximately 20 µm in diameter. Part of our work in the future will be to push our measurement capabilities to smaller particles.

The third material investigated is a thin (~200 nm) film of polycrystalline $BaTiO_3$ deposited on MgO. The $BaTiO_3$ is oriented such that, for each film, either the c-axis or the a-axis is normal to the substrate. Some of the films appeared to be a mix of the two orientations, and the question was whether the ratio of the two orientations was the same across the film. The 30 µm beam collimator was used to sample areas across the film, and a difference in the profile of the 200/002 peak was observed, indicating a change in the ratio of the two orientations.

We plan to continue our work by improving the sensitivity of the technique, testing minimum particle size/mass requirements, and investigating the use of focussing capillaries as well as applying the technique to technological materials of interest.

21. Development of Standards for Optical Particle Counters

R.A. Fletcher, J.R. Verkouteren, E.S. Windsor, J.A. Small, D.S. Bright, E.B. Steel and W.S. Liggett (882)

Objective: To provide a Standard Reference Material (SRM) for calibrating optical particle counters (OPCs) that measure particulate contamination in hydraulic fluids. The National Fluid Power Association (NFPA) asked NIST to develop a material that would provide national (NFPA/T2.9.1.1 R1-19xx) and international (ISO 4402:199x) traceability for contamination measurements. SRM 2806 and RMs 8631 and 8632 were developed for these purposes.

Problem: Optical particle counters, which make very precise measurements, are widely used by the fluid power industry to monitor the particle contamination of fluids and to test the capacities and characteristics of filters used in hydraulic systems and vehicles. For the past 25 years, Air Cleaner Fine Test Dust (ACFTD - a polydisperse mineral dust), has been used as a *defacto* particle standard under the guidelines of ISO Method 4402. Need for a new particle size calibration SRM is driven by the unavailability of ACFTD and recognition by the Fluid Power Community that the reference particle size distribution for ACFTD in the current ISO document is inaccurate.

Approach: A multi-step certification process was developed to quantify the precision and accuracy associated with the particle size distribution of SRM 2806. SRM 2806, composed of ISO Medium Test Dust (a derivative of Arizona Road Dust), was suspended in MIL-H-5606 hydraulic fluid batches by Fluid Technology Inc. As a first step in the certification procedure we determined the within-and between-batch homogeneity using a precise OPC. Accuracy of the size distribution, however, is derived from particle size measurement by microscopy. The mineral particles were quantitatively extracted from a known volume of fluid and digital images of the extracted particles were collected using a scanning electron microscope operating in the electron backscatter mode. Special care was taken to assure random sampling from the filter surface and proper microscope, magnification calibration using SRM 484f. The digitized images were processed individually to obtain particle size and number. Measurements were, and will continue to be, made to monitor the SRM shelf life/stability. Material made to the SRM specification was subjected to an international round robin test. SRM 2806 has also undergone a U.S. round robin.

The two Reference Materials, RM 8631 (ISO Medium Test Dust) and 8632 (ISO Ultra Fine Test Dust), were donated to NIST by Particle Technology Inc. Both materials were split into small, 20-g aliquots and tested for batch homogeneity using a calibrated optical particle counter. A U.S. round robin was also conducted using both materials.

Results and Future Plans: The measurements determining the homogeneity of SRM 2806 indicate that the material has a within-batch relative expanded uncertainty of less than 1% for particle diameters below 5 µm and 6% for the largest particles which are greater than 30 µm. The batch-to-batch homogeneity measurements have a relative standard deviation of approximately 2% for particles less than 5 µm and 7% for particles greater than 30 µm. Certification of one batch of material is nearly complete. As expected, the size distribution determined by microscopy contains many more particles in the small size fraction than the distribution previously specified for the ACFTD. Since there are some batch-to-batch differences, future work will entail certifying each batch of SRM material and monitoring its stability. SRM 2806 and RMs 8631 and 8632 will be available for use during the spring of 1997.

22. *Transient Enhanced Diffusion in Low Energy Boron-implanted Silicon*

D.S. Simons, P.H. Chi, K.S. Jones (Univ. of Florida), and L.H. Zhang (Univ. of Florida)

Objective: To measure the diffusion of low energy ion-implanted boron in silicon under typical annealing conditions for semiconductor processing so that more accurate process modeling can be achieved.

Problem: As semiconductor device dimensions decrease, the need to produce shallow junction depths requires that ion implantation of dopant species be made at reduced energies. In crystalline materials, ion channeling effects can produce extended tails of dopant depth distributions. A pre-amorphization implant of Si^+ or Ge^+ is commonly employed to eliminate the channeling. During the annealing that heals the implant damage, the dopant may diffuse deeper into the silicon substrate producing a deeper junction than desired. The diffusivity of boron in silicon is much greater than expected during the early stages of annealing following implantation. This phenomenon is known as transient enhanced diffusion (TED). Process modelers must understand the detailed boron motion under these conditions to develop accurate process models for advanced devices. An accurate characterization of TED is essential for understanding the physical process involved and establishing conditions to minimize adverse effects.

Approach: We have undertaken a study of boron diffusion in ion-implanted silicon by a combination of secondary ion mass spectrometry (SIMS) and transmission electron microscopy (TEM). B^+ was implanted at an energy of 4 keV with a dose of $1x10^{14}$ cm^{-2} into either (100) Si or Si that had been preamorphized by Ge^+ implantation at 180 keV at a dose of $1x10^{15}$ cm^{-2}. These implants were subsequently annealed in a furnace with a nitrogen atmosphere at 800 °C for times ranging from 4 min to 4 h. Depth profiles of the boron distribution were made by SIMS using low energy O_2^+ bombardment and O_2 flooding of the sample surface to minimize redistribution artifacts during the profiling. TEM micrographs were made of the same samples to correlate the observed defects with the TED.

Results and Future Plans: For the B^+ implants in crystalline Si, TED of the implant tail was observed in SIMS profiles for anneal times as short as 4 min, but no difference was seen for 15 min to 2 h anneals. The peak region of the implant is largely immobile, as has been observed by others. This is possibly due to a high concentration of boron-interstitial clusters that do not diffuse as readily as isolated boron atoms. In the preamorphized sample, the behavior of boron is quite different. The as-implanted profile is much shallower due to the absence of channeling. Upon annealing, the boron peak is not immobile and the profile continues to broaden with increasing time to the maximum at 4h. The estimated diffusivity decreases with time, from a value 50x larger than the normal value over the first 4 min, to only 5x the normal value after 11 min. Associated TEM studies show that (311) defects and dislocation loops are present in a narrow zone at a depth of 230 nm representing the end-of-range damage of the Ge implant. Most of the defects dissolve over 1h of annealing, suggesting that the (311) defects are the source of interstitials that drive the TED in this case. The data produced in this study will be made available to the modeling group at SEMATECH for incorporation of it into their advanced process models.

A planned future study will test the hypothesis that the immobility of the boron peak in crystalline Si is due to clustering. Controlled excess interstitials will be introduced by different doses of Si implantation to test the effect on boron redistribution.

Publications:

Jones, K.S., Zhang, L.H., Krishnamoorthy, V., Law, M., Simons, D.S., Chi, P., Rubin, L., and Elliman, R.G., **"Diffusion of ion implanted boron in pre-amorphized silicon,"** Appl. Phys. Lett. 68, 2672-74 (1996).

IV. Physical and Chemical Properties Division (838)

Richard F. Kayser, Chief

A. Division Overview

Recent History:
The Physical and Chemical Properties Division was created in the spring of 1996 when

- the Pressure and Vacuum Group was moved from the old Thermophysics Division to the Process Measurements Division (PMD)
- the remainder of the old Thermophysics Division and the old Chemical Kinetics and Thermodynamics Division were combined into the new division.

The first change consolidated in the PMD most of CSTL's programs in basic physical measurements, standards, and services. The second change consolidated CSTL's data-oriented programs in thermophysics, thermochemistry, and chemical kinetics.

Mission:
The Physical and Chemical Properties Division is the Nation's reference laboratory for measurements, standards, data, and models for

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

The Division develops and maintains state-of-the-art experimental, theoretical, and modeling capabilities and provides Standard Reference Data, Standard Reference Materials, calibrations, and technical research and services to promote U.S. economic growth and to assist U.S. industry, other government agencies, and academia in solving problems of national importance.

Functions:
In the areas of thermophysics, thermochemistry, and kinetics, the Division

- develops, maintains, and uses state-of-the-art apparatus to perform experimental measurements on industrially and scientifically important systems
- compiles, evaluates, and correlates experimental data
- develops and evaluates state-of-the-art theories, models, estimation methods, and computational approaches
- disseminates results by a wide variety of mechanisms.

The Division also develops advanced cryogenic technologies and provides the Nation with measurement standards and services for flow under cryogenic conditions.

***Current Focus Areas*:**
Current focus areas include *Basic Reference Data, Data for Process and Product Design*, *Properties of Advanced Working Fluids*, *Environmental Fates of Industrial Chemicals*, *Tools for Chemical Analysis*, *Studies of Complex Fluids, and Cryogenic Technologies.* Brief descriptions of these areas follow with references to Selected Technical Reports (which present a cross section of activities, not a complete description).

<u>Basic Reference Data</u>
Physical, chemical, and kinetic data and models are important in a wide range of contexts and applications, including research and development, process modeling, process design, energy efficiency, safety, health, transportation and storage, custody transfer, and the environment. The principal components of this program are

- to compile, evaluate, and disseminate data and predictive models for properties and chemicals of broad interest to a large number of diverse users; see Reports 1-3
- to maintain, develop, and apply experimental and computational capabilities for acquiring such data; see Reports 4-8.

High-priority needs include sufficient data for many substances to determine parameters in commonly used models; data to develop new and improved estimation methods and models; and data to provide a common basis for evaluating and intercomparing estimation methods, models, and computational techniques.

Data for Process and Product Design

Process and product design in a wide variety of industries depends on the availability of high-quality physical and chemical properties data and models. The availability of such information has played a key role in the emergence of process simulators as a key enabling technology in the chemical and related industries. Large-scale process modeling and simulation is also growing in importance in areas such as semiconductor processing and the treatment of hazardous wastes. The Division's goal is to provide the underlying data and models needed to support such developments. Areas of current interest include

- data for separations – distillation, extraction, adsorption, and membrane separations; see Reports 7 and 9-11
- thermophysical properties and chemistry of semiconductor processing gases; see Report 5
- data for advanced oxidation technologies; see Reports 12-13

Properties of Advanced Working Fluids

Accurate and comprehensive equilibrium and transport properties data and models for fluids and fluid mixtures over wide ranges of conditions are essential to design and optimize the performance of working cycles in refrigeration systems and power plants. Efforts in this area include the acquisition of the required data and the development and dissemination of high-accuracy correlations. Fluids under study include

- alternatives to the ozone-depleting CFCs (chlorofluorocarbons) and HCFCs (hydrochlorofluorocarbons) for use in air-conditioning and refrigeration systems; see Reports 14-15
- noble gas mixtures for use in thermo-acoustic refrigerators; see Report 5
- water and steam for use in conventional power plants; see Reports 16-17
- ammonia-water mixtures as alternatives to steam in applications ranging from direct-fired power plants with high operating temperatures to geothermal power plants with relatively low operating temperatures; see Report 18.

Environmental Fates of Industrial Chemicals

A wide variety of physical, chemical, interfacial, and kinetic data and data prediction methods are essential to understand and assess the long-term fates of chemicals in the environment. Questions of great import to industry, the Nation, and the world range from the fate of new and existing chemicals in the atmosphere to the fate of hazardous chemicals and toxic metals in water and the ground. Properties of interest include atmospheric lifetimes, aqueous solubilities, ion-exchange equilibria, and partition coefficients. Current efforts are focusing on:

- the atmospheric chemistry and lifetimes of alternative refrigerants, alternative fire suppressants, and oxygenated fuel additives, including the chemistry of atmospheric aerosols; see Report 19
- the thermodynamics and kinetics of inorganic and organic species in aqueous solutions; see Reports 17 and 19.

Tools for Chemical Analysis

The analysis of simple and complex mixtures and the identification of individual chemicals are tasks central to many aspects of the chemical and related industries, including research and development, production, and quality control and assurance. These tasks are also of crucial importance in other industries and in areas such as environmental monitoring, medical research, and health care. Here the Division strives to produce critically evaluated data, predictive models, and software to assist in the analysis and identification of as wide a range of compounds as possible. Areas of current emphasis include

- mass and infrared spectral data and databases; see Reports 20-21
- chromatographic data and databases; see Reports 22-23.

In addition to the products described in Reports 20-23, all of this information will appear in the NIST Chemistry WebBook; see Report 1.

Studies of Complex Fluids

The goals of this program are to develop and use unique experimental, theoretical, and simulational capabilities to study complex fluid systems under equilibrium and non-equilibrium conditions, with and without chemical reactions. As indicated in Report 24, current efforts include studies of

- the equilibrium microscopic structure of colloids, gels, micelles, and emulsions

- the effects of processes such as shear on the microstructure of such systems and on the dynamics of processes such as coagulation, gelation, and association.

This work is related to the "intelligent processing of materials", in which one tries to relate the microscopic structure of a fluid (or other material) during processing to its final bulk properties and to control the process accordingly.

Cryogenic Technologies

Cryogenic technologies underpin a wide variety of technically important areas, including the cooling of electronics for high-speed computing and communications, the production of ultra-clean environments for semiconductor processing, the liquefaction of natural gas from remote gas wells and for use as a clean fuel, the cooling of medical instruments, and the cooling of satellite-based infrared sensors for military and environmental applications. Research in this program addresses all of these topics, with the primary focus on the development of new and improved cryocooler processes, especially pulse-tube refrigerators, capable of operating at temperatures from 120 K to below 10 K. The program encompasses the development of improved measurement and modeling techniques for characterizing cryocooler components and improving their performance. Other areas of emphasis include research on cryogrinding and on methods of measuring cryogenic flows. See Report 25.

Future New Thrusts:

Computational Chemistry

The Division has initiated a new program in computational chemistry that will consolidate current efforts and build upon the Division's traditional strengths in the area of data. The goal is to create the NIST Computational Chemistry Resource (CCR) to aid industrial scientists and engineers in the computation of molecular properties; see Report 26. The CCR will contain the following three elements:

- critical comparisons of computational results with the best available experimental data
- interactive guidance for the non-expert in the choice of methods, reliability, and resource requirements
- a searchable repository where scientists may submit or extract the results of standard calculations.

This program will address the needs identified in the 1993 NIST Workshop on Industrial Applications of Computation Chemistry, the 1996 American Chemical Society Symposium on Computational Thermochemistry (co-organized by NIST and The Dow Chemical Company), and Technology Vision 2020: the Chemical Industry Roadmap.

Selected Technical Highlights:

- development and release of the first version of the NIST Chemistry WebBook (Report 1)
- development of new measurement capabilities for the properties of fluids: Greenspan viscometer (Report 5); vapor-liquid-equilibrium apparatus and total-enthalpy flow calorimeter (Report 7)
- development and use of cavity-ring-down spectroscopy to measure gas-phase reaction rates (Report 8)
- development and release of new version of the NIST/ASME Thermophysical Properties of Water Database (NIST STEAM) based on the new international standard and incorporating a Windows™-based graphical user interface (Report 16)
- completion of a comprehensive data compilation and evaluation effort for the thermophysical properties of ammonia-water mixtures and of an interim model for that system (Report 18)
- completion of the first version of the NIST Automated Chemical Identification System (ACIS) as part of efforts to identify chemical weapons (Report 21)
- discovery of a dynamic scaling relation for the microscopic structure of systems undergoing gelation (Report 24)
- development of a large pulse-tube refrigerator for liquefying natural gas as part of a cooperative research and development agreement with Cryenco, Inc. (Report 25).

Organizational Structure:

The Physical and Chemical Properties Division has research groups in both Gaithersburg, Maryland and Boulder, Colorado, with the equivalent of approximately 35 full-time employees in each location. **Dr. William M. Haynes** directs the overall efforts of the Boulder part of the Division. The structure and technical activities of the Division are as follows:

Division-Office Programs (Gaithersburg and Boulder)

- develops models and correlations for fluids and fluid mixtures
- uses unique experimental, theoretical, and simulational capabilities to study complex fluids under both equilibrium and non-equilibrium conditions
- develops and applies techniques for calculating condensed-phase properties and behavior using molecular dynamics and Monte Carlo simulation techniques
- develops thermochemical and spectral databases and data prediction methods

Process Separations Group (Boulder)

- performs basic and applied research on a variety of separation processes, including distillation, supercritical fluid extraction, adsorption, and membrane separations
- provides critically-evaluated data and models needed to design and/or select more efficient and robust separation processes.

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, estimation methods, and computational techniques for thermo-dynamic properties and rate constants
- compiles, evaluates, correlates, and disseminates Standard Reference Data and develops and disseminates electronic databases and software on chemical kinetics in the gas and liquid phases; the chemistry and thermochemistry of industrially and environmentally important chemical species; and analytical mass and infrared spectra.

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 fluid 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, hydrocarbons, refrigerants, and aqueous systems

Theory and Modeling of Fluids Group (Boulder)

- performs theoretical and simulational 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 advanced measurement techniques, standard measurement practices, and mathematical models for cryogenic processes and develops new methods and systems for producing cryogenic temperatures
- assists U.S. industry in the development of products utilizing cryogenic processes

Staff Recognition for Fiscal Year 1996:

- Donald G. Archer, 1996 Stig Sunner Memorial Award of the Calorimetry Conference for his correlation of the dielectric constant of water, work on the thermodynamic properties of salt and surfactant solutions, and enthalpy-increment measurements on important substances, all over wide ranges
- Malcolm W. Chase, Department of Commerce Bronze Medal for making NIST Standard Reference Data available as electronic databases, expert systems, and other software
- Allan H. Harvey, NIST Measurement Service Award for the development of a new database for the thermophysical properties of water and steam based on the new international standard
- Richard F. Kayser, Department of Commerce Silver Medal for technical and managerial leadership as chief of the Physical and Chemical Properties Division of NIST
- Michael J. Kurylo, NASA Exceptional Service Medal for outstanding leadership of NASA's Mission to Planet Earth's Upper Atmosphere Research Program (UARP); 1996 Catholic University of America Alumni Achievement Award in the Field of Science for important contributions to chemical kinetics related to the earth's atmosphere
- Rhoda D. Levin, 1996 NIST Equal Employment Opportunity Award for leadership and extensive contributions to the NIST Mentoring Program and the NIST Diversity Board
- Mark O. McLinden, National Society of Professional Engineers Engineer of the Year for the Department of Commerce for outstanding contributions to national and international efforts to replace chlorofluorocarbons with non-ozone-depleting alternatives in air-conditioning and refrigeration equipment
- Laurell R. Phillips and Carol A. Thomas, Special Act Awards for providing outstanding support for two divisions after the loss of a division secretary and during a lengthy transition to a single new division
- Jan V. Sengers and John S. Gallagher, Certificates of Recognition from the American Society of Mechanical Engineers for contributing greatly to the development and implementation of improved technologies in the field of utility and industrial steam generation
- Johanna M.H. Levelt Sengers, Election to National Academy of Sciences for her outstanding research on the physics and chemistry of fluids and fluid mixtures over wide ranges of conditions, including the critical region.

B. Selected Technical Reports

1. Dissemination of NIST Reference Data over the Internet: The NIST Chemistry WebBook

__W.G. Mallard,__ P.J. Linstrom (Contractor), Steve Stein, P.J. Christian, J.F. Liebman (Univ. Maryland, Baltimore Campus), and Hussain Affefy (UMBC)

Objective: To provided Internet access to a broad range of chemical data with a common interface that provides both ease of use and a simple method to expand as more data become available.

Problem: There is an enormous amount of data on chemical compounds that has been measured, collected, and in many cases evaluated, but it is largely inaccessible with electronic data retrieval. In particular, many of these data appear in printed monographs, many of which are hard to find, out of print, or woefully incomplete. Primary goals of this project are (a) to find, and if needed, evaluate these data, and (b) to provide the mechanisms needed to make these data as well as all NIST chemical reference data available on the Internet.

Approach, Results, and Future Plans: During FY 1996, the first version of the NIST Chemistry WebBook* was released. The initial data sets were those originally contained in the Structures and Properties and Ion Energetics Databases. This data set consisted of thermodynamics data for over 5000 compounds and ion energetics data for over 7000 compounds; it is searchable by name, molecular formula, and partial molecular formula. The WebBook has been very well received, and usage has grown greatly both in number of users and number of repeat users. The number and variety of users – from industry, government and academia – is a clear indication of the need for this type of service. The next release of the WebBook, scheduled for early 1997, will add infrared spectra, mass spectra, vapor-pressure data, and data on heat capacity of liquids, and it will greatly increase the number and quality of the thermochemical and ion energetics data, including the evaluated proton affinity data. Many of the other projects discussed below will also feed data into the WebBook. A long-range goal for this project is to make the NIST WebBook the source of first choice for chemical and physical property data.

2. Evaluated Thermodynamic Data for Neutral and Ionic Species

__M.W. Chase__, E.P. Hunter, and S.A. Crisen

Objective: To develop reliable evaluated thermochemical data needed for both neutral and ionic species that are critical to the technical community.

Problem: Thermochemical data for a wide variety of compounds important in modern technology are either not available or are of such poor quality that the use of modern modeling techniques, necessary to improve process control or overall efficiency, is not possible. In many of the most important cases, a critical review of all of the available data on a particular species can significantly reduce the uncertainty of the data, and therefore enhance the accuracy of the models in which the data are used.

Approach: The literature data are critically assessed. All of the available data, both thermochemical and spectroscopic, are examined. Multiple determinations are not common so data must be compared to data for similar systems. In addition, the pertinent references are documented, and where applicable, computations of thermodynamic functions as a function of temperature are performed from first principles. In all cases, comparisons are made between the data and the results of various predictive methods. In the case of ion thermochemistry, there is an additional need to resolve the data for complex thermochemical ladders.

Results and Future Plans: There have been two distinct components of this work during the past year. The first focuses on the thermochemistry of neutral species, the second on ionic species. While the goal of producing accurate heats of formation for species is the same for both components, the experimental methods for the two areas are very different. The neutral thermochemistry is focused on producing high-quality, temperature-dependent data for a number of atmospheric species, and for species that are important in processes involving halogens. Additional work is proceeding on organic compounds of interest to kinetic modelers (20 compounds). The ionic work is focusing

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* http://WebBook.nist.gov

on providing a consistent proton affinity scale, with far less concern with temperature dependence. This scale is of central importance in determining the interactions and equilibria of ionic species in solution. These data cannot be measured directly and must be painstakingly evaluated from many experimental and theoretical studies; in fact, *ab initio* calculations were used extensively to resolve experimental discrepancies that have revolved around the proton affinity scale for the past several years. The new proton affinity scale will be published in early 1997.

Publications:

Chase, M.W., ***"NIST-JANAF Thermochemical Tables for the Oxygen Fluorides,"*** J. Phys. Chem. Ref. Data 25, 551 (1996).

Chase, M.W., ***"NIST-JANAF Thermochemical Tables for the Bromine Oxides,"*** J. Phys. Chem. Ref. Data 25, 1069 (1996).

Chase, M.W., ***"NIST-JANAF Thermochemical Tables for the Iodine Oxides,"*** J. Phys. Chem. Ref. Data 25, 1297 (1996).

3. Critical Evaluation of Chemical Kinetics Standard Reference Data

W. Tsang, R.F. Hampson, W.G. Mallard, V.I. Babushok (Russian Acad. Sciences, Russia), D.H. Frizzell, J.J. Reed, F. Westley (Contractor), Y.A. Mirokhin (Inst. Avionics Equipment, Russia), J.T. Herron (Contractor), and C-Y Lin (Contractor)

Objective: To provide consistent sets of rate constants (and the associated thermochemical data) for important reaction systems.

Problem: To understand the complex chemical processes in the atmosphere, a furnace, or a chemical vapor deposition reactor, it is often necessary to create a model of the detailed chemical changes that are occurring within the system. To do this, a rate for each reaction in the system is needed, usually as a function of temperature. While many of these data exist, they are often fragmentary and difficult to find. In addition, older analyses of experiments have often proven to be incomplete, and the resulting rate constants reported incorrect.

Approach: The Kinetics Data Center collects and evaluates data on the rates of gas-phase chemical reactions that are needed for modeling natural and industrial processes. There are several components of this work. First, the technical literature is routinely scanned and all publications presenting data on the rates of chemical reactions are retrieved, data on all neutral gas-phase reactions are abstracted, and data relevant to specific evaluation projects are forwarded to the evaluators. The evaluators then compare the data to data from other similar systems, and when possible, model the entire complex system using the best available rate constants. These model results are then compared to the experimental results. The resulting kinetic parameters are examined to determine trends in reactivity and associated bond energies implied by the kinetic parameters. Comparisons of the results to theoretical calculations are often performed. The results of these studies are published in the literature and distributed in the form of electronic data sets. All abstracted data, even those not involved in a current evaluation project, are incorporated into the NIST Chemical Kinetics Database.

Results and Future Plans: Two areas in which kinetic data are especially important are atmospheric chemistry and combustion. These have been the focus of evaluations for several years. One interesting new development has been the work on flame inhibition. This has led to the publication of an extensive review of data for fluorinated hydrocarbons (also available on the Internet; see below). Some of the complex reactions in the combustion of larger hydrocarbons have required more powerful computational tools for their analysis. This has led to the development of master-equation solutions to complex thermal decompositions. As the need to analyze more complex molecules grows, these tools will become indispensable in untangling the complexities of large molecular reactions. The ongoing work with IUPAC and the NASA Upper Atmosphere evaluation processes has been extended to include initial data on sulfates. Finally, the currently released version of the NIST Chemical Kinetic Database (6.01) contains data relating to over 8800 reactant pairs. A new Windows version of the program and an extensive new set of data will be available early in 1997.

Publications:

Babushok, V.I., Noto, T., Burgess, D.R.F., Hamins, A., Tsang, W., and Miziolek, A., ***"Effect of Halogenated Flame Inhibitors on C_1-C_2 Organic Flames,"*** in Proc. 26th Symposium on Combustion (in press).

Burgess, D.R.F., Zachariah, M.R., Tsang, W., and Westmoreland, P.R. ***"Thermochemical and Chemical Kinetic Data for Fluorinated Hydrocarbons,"*** Prog. Energy Combustion Sci. 21, 459 (1996). On the internet,
see http://fluid.nist.gov/836.03/papers/NistTNIntro.html

Tsang, W., Bedanov, V. and Zachariah, M.R., ***"Master Equation Solution of Thermal Activation Reactions: Energy Transfer Constraints on Fall-off Behavior in the Decomposition of Reactive Intermediates with Low Thresholds,"*** J. Phys. Chem. 100, 4011 (1996).

4. *Ab Initio Prediction and Modeling*

K.K. Irikura, R.D. Johnson III, W.J. Stevens, and P. Hassanzadeh (George Washington Univ.)

Objective: To generate and evaluate chemical thermodynamics and kinetics data using modern methods of computational chemistry, and to develop and evaluate *ab initio* methods for the prediction of molecular energetics and partition functions.

Problem: Accurate thermodynamics and kinetics data are required for successful modeling of atmospheric chemistry, semiconductor processing, industrial chemistry, and many other processes of great economic importance. Unfortunately, the necessary data are often missing or of unknown reliability, destroying the credibility of affected models. Moreover, data needs often overwhelm experimental resources. NIST is trying to meet this data requirement.

Approach: *Ab initio* methods of quantum chemistry are sufficiently mature to provide acceptable data for large classes of molecules. In many cases, experimental data for related species can be exploited to produce very reliable, precise predictions. Current efforts are focused on thermochemistry and kinetics, for which the relevant quantities are molecular energetics (enthalpies) and molecular energy levels (entropies and heat capacities). Available methods are used to produce calculated results for specific molecules. Contributions to the current state of the art for computations are through the development and evaluation of such methods.

Results and Future Plans: Most of the effort is directed toward molecules that present unusual experimental difficulties, such as reactive transients or compounds of high toxicity. In the past year, completed work includes calculational studies related to atmospheric chemistry (iodine oxides, N_2O_5, and fluoroalkyl radicals), semiconductor processing (silicon- and boron-containing radicals and ions), combustion modeling (the hydroxymethyl radical), and DNA damage (several oxyradicals), and to evaluation of several methods for predicting vibrational anharmonicities (contribution to current art). Current and future work includes more studies such as those listed, and also prediction of Benson-type group values, development of methods for molecular energetics, and computation of reaction rates. Collaborations, including several within NIST and with The Dow Chemical Company and Brookhaven National Laboratory, have been very helpful and will be increasingly important as work progresses.

Publications:

Atkinson, D.B., Irikura, K.K., and Hudgens, J.W., *"**Electronic Structure of the BF_2 Radical Determined by ab Initio Calculations and Resonance Enhanced Multiphoton Ionization Spectroscopy,**"* J. Phys. Chem. (in press).

Hassanzadeh, P. and Irikura, K.K., *"**Nearly ab Initio Thermochemistry: The Use of Reaction Schemes. Application to IO and HOI,**"* J. Phys. Chem. (in press).

Johnson III, R.D., Hudgens, J.W., and Ashfold, M.N.R., *"**Excited Electronic States of the SiF_2 Radical Studied by Resonance-Enhanced Multiphoton Ionization Spectroscopy and ab Initio Methods,**"* Chem. Phys. Lett. 261, 474 (1996).

Johnson III, R.D. and Hudgens, J.W., *"**Low-lying Vibrational States of Hydroxymethyl (CH_2OH) Radicals and Cations Derived from Observations of Electronic Spectra and from ab Initio Calculations,**"* J. Phys. Chem. (in press).

5. *Greenspan Acoustic Viscometer for Property Measurements in Gases*

K.A. Gillis, J.B. Mehl (Univ. Delaware), and M.R. Moldover

Objective: To develop a robust instrument for measuring the viscosity of gases, including the reactive and toxic gases used in semiconductor processing and the noble gas mixtures used in thermoacoustic refrigerators.

Problem: Accurate data for transport properties of gases are needed to rationalize the metering of gases and to optimize the design of heat exchangers using gases. Instruments for measuring the transport properties of gases are fragile and expensive to use. Acoustic instruments have the potential of being robust and accurate under a wide range of conditions; however, sophisticated algorithms are required to interpret the data. NIST is optimizing the design of these instruments and developing algorithms for interpreting the data obtained with them.

Approach: For measuring the viscosity of gases, we have developed the Greenspan acoustic viscometer. This compact device is consist of two cavities connected by a long narrow duct. A typical cavity has a volume of 30 cm^3; a typical duct is 6 cm long and has a radius r_d = 1 nm to 3 mm. The gas oscillates at frequency f between two chambers through the duct. In a first approximation, the viscosity η of the gas is related to the quality factor Q of the oscillation by $Q^2 = r_d^2\,\rho\pi f/\eta$, where ρ is the mass density of the gas. As developed by us, the Greenspan viscometer is an absolute instrument; that is, the quantity η/ρ is deduced from measurements of the dimensions of the instrument and its frequency response. No calibration is used.

Results and Future Plans: At the time the article listed below was written, our "best" Greenspan viscometer had a systematic error component approximately 1% of the observed value. Subsequently, the theory of the instrument was improved and four new viscometers were manufactured. Two have ducts with radii of 1.0556 mm and two have ducts with radii of 2.3141 mm. New test data were taken for argon at ambient temperature and spanning 2½ decades in the quantity η/ρ. The deviation plot compares the new results with the literature on an absolute basis. The relative mean and RMS deviations of the data taken with the 2.3 mm resonators are 0.002 and 0.003 respectively and -0.005 and 0.003 respectively. The relative mean and RMS deviation of the data taken with the 1.0 mm resonator are 0.001 and 0.006 respectively and -0.002 and 0.003 respectively. It is remarkable that the instruments with different values of r_d and ducts of different lengths give comparably good results. When the test gases were helium and propane, the agreement with data from the literature was equally as good.

A "production" viscometer is now being constructed. The parts of this new viscometer that contact the test gases will be made entirely of stainless steel and gold. This instrument will be integrated into a system that controls the temperature and pressure; thus, accurate measurements will be possible with a wide range of gases under a wide range of conditions at reasonable cost.

A new acoustic resonator is being designed to measure the Prandtl number [$Pr \equiv$ (viscosity × heat capacity)/(thermal conductivity)], which is the key parameter governing heat transfer from a solid to a flowing gas. For measuring the Prandtl number, NIST developed a cylindrical resonator with an insert comprised of an array of plates parallel to the cylinder axis. Typically, the array is located near the middle of the resonator and is 1/10 of the length of the resonator. The motion of the test gas in the vicinity of the array causes large losses, either from the viscosity of the gas or its thermal conductivity, depending upon the resonance mode chosen. The Prandtl number is deduced from measurements of the frequency response of the resonator in the vicinity of several modes. The analysis does not require accurate, difficult-to-make dimensional measurements of quantities such as the area of the array.

Publications:

Gillis, K.A., Mehl, J.B., and Moldover, M.R. ***"Greenspan Acoustic Viscometer for Gases,"*** Rev. Sci. Instrum. 67, 1850-1857 (1996).

6. *Primary Acoustic Thermometry at High Temperatures*

***D. Ripple** (Process Measurements Division), M.R. Moldover, and K.A. Gillis*

Objectives: (1) To reduce the uncertainty in the determination of the thermodynamic temperature by a factor of 3-8 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 shall measure the frequencies of both acoustic and microwave resonances in a spherical, argon-filled cavity bounded by a thick, metal shell which in turn will be enclosed by a high-performance thermostat. The data will determine the speed of sound in the argon from which the thermodynamic temperature will be deduced. The temperature will be transferred to platinum resistance thermometers and then to fixed-point devices. For acoustic thermometry, the measured quantities are frequencies and the temperature. This contrasts with CVGT which requires the measurement of pressures and temperature; thus, systematic effects will be very different in the present work and may resolve the discrepancies in the CVGT.

Results and Future Plans: A significant technical challenge was to develop electro-acoustic transducers that are compatible with the high temperatures and which neither contaminate the argon nor significantly perturb the resonance frequencies. During FY 1996, we developed a novel capacitance microphone with a silicon "plate" that moves in response to the sound field, electrostatic forces applied by a counter electrode, and the impedance of argon in a cavity behind the plate. This microphone does not have a linear frequency response; however, its frequency response can be accurately modeled. In addition to functioning at the temperatures and pressures required, this microphone is made of gold, stainless-steel, and silicon; thus, it will not contaminate the test gas.

During FY 1997, a spherical cavity will be set up together with a gas handling system. They will function as a test bed for studying the influence of flow on the resonance measurements and for characterizing transducers at ambient temperatures. We will construct the high-temperature resonator and the high-performance thermostat.

7. *New Systems for Measuring the Thermophysical Properties of Fluids and Fluid Mixtures*

***R.A. Perkins**, T.J. Bruno, C.D. Holcomb, A. Laesecke, B. Louie, J.W. Magee, M.O. McLinden, S.L. Outcalt, L.A. Watts, J. Blanco (Univ. Colorado), M. Cremer (Univ. Hannover, Germany), B.N. Hansen (Univ. Colorado), T. Lüddecke (Univ. Hannover, Germany), E. Malkin (Weizmann Institute, Israel), and C. Muzny (Univ. Colorado)*

Objective: To develop new experimental measurement capabilities for the thermophysical properties of fluids and fluid mixtures to enable the U.S. to address critical problems requiring properties.

Problem: Thermophysical-properties data are vital for a wide range of applications, including process simulation in the chemical process industries and equipment simulation and design in the air-conditioning, refrigeration, and power industries. Accurate property data and models based on these data are the key to generating results that can be used with confidence. These data are required for an ever-widening variety of fluids and fluid mixtures over wide ranges of temperature, pressure, and composition.

Approach: The development of experimental measurement capabilities for the thermophysical properties of fluids has been supported by the Department of Energy (DoE) and NIST for many years. State-of-the-art measurements carried out on apparatus developed under DoE sponsorship have been the cornerstone of our programs on the alternative refrigerants, natural gas, and petroleum systems. These measurements also provide the data which are needed to develop the models which underlie our Standard Reference Databases.

Results and Future Plans: A versatile apparatus has been developed to measure vapor-liquid phase equilibria (VLE), saturated liquid and vapor densities, and interfacial tension at temperatures from 200 to 425 K. This apparatus has been calibrated and its performance has been verified. It is now in routine use for accurate measurements of mixtures of refrigerants.

A total-enthalpy flow calorimeter has been developed which measures the enthalpy change, at constant pressure, of a fluid over a temperature range from 200 to 500 K. It allows the measurement of isobaric (constant pressure) heat capacity and the enthalpy of vaporization. The design target for the relative standard uncertainty of the instrument is ±0.1%, which will be verified in the coming year with measurements on refrigerants.

Development of a vibrating-wire viscometer cell has been completed for measurements at pressures to 70 MPa. An associated cryostat that will cover the temperature range from 80 to 500 K is nearing completion. A second vibrating-wire viscometer is also nearing completion for studies of ammonia/water mixtures at temperatures to 700 K. The high-temperature cell of this system is enclosed in a furnace and has a small cell volume to facilitate measurements on hazardous or scarce samples. Both of these instruments can be operated in steady-state or transient modes for studies of a wide range of fluids, including polar and electrically conducting fluids.

An apparatus has been developed to measure the vapor-liquid equilibria and the densities of the liquid and vapor phases for azeotropic aqueous-organic mixtures that can be broken by the addition of a salt. The calibration of supporting instrumentation is underway. Procedures are being developed for reliable sampling and compositional analysis of the phases. Performance testing will be completed during FY 1997 for mixtures of ethanol, water, and NaCl.

A low-angle light-scattering apparatus is under development to measure the thermal diffusivity and to determine the critical point parameters of fluids and fluid mixtures. The present measurement cell has a small volume (<5 cm^3) which can be used at temperatures from 250 to 400 K with pressures to 20 MPa. The performance of the apparatus is currently being tested with carbon dioxide. During the coming year, the optical design will be refined and a corrosion resistant measurement cell will be constructed for use at temperatures from 80 to 550 K at pressures to 70 MPa.

An apparatus is being developed to measure the solubility of solids in dense (or supercritical) fluids. In this apparatus, a solute is suspended in a dissolution chamber by a coaxial magnetic suspension which allows force measurements to be made. Solvent is then added to the vessel, and solute leaves the buoy to enter solution. The mass change of the buoy, appropriately corrected, allows calculation of the solubility of the solute. This approach is especially valuable for mixtures that cannot be analyzed by conventional means such as spectrophotometry or chromatography.

A small-volume, dual-cell, dew-bubble point apparatus is in the initial stages of development and is designed to operate over a wide range of temperatures and pressures. The apparatus will measure with a high level of accuracy the vapor pressures of pure fluids and the dew and bubble point pressures of standard mixtures. Because of its small volume, the apparatus will be especially useful for studying fluids which are expensive, toxic, corrosive, or have a limited availability.

Development of a heat-of-vaporization calorimeter / effusion cell will begin this coming year for accurate determination of the vapor pressures of fluids below their normal boiling point. This technique will complement direct measurements of the vapor pressure which are subject to increasing uncertainty at low pressures.

8. *Cavity-Ring-Down Technology for Kinetic and Mechanistic Studies*

***J.W. Hudgens**, R.E. Huie, D.B. Atkinson, and A.C.R. Pipino*

Objectives: To improve the accuracy, precision, and sensitivity of optical absorption measurement protocols and to use these new protocols to measure chemical reaction rates in the gas phase, in condensed phases, and at interfacial thin films.

Problem: The development of accurate computer models of waste incineration, semiconductor processing, atmospheric chemistry, and solution chemistry will require accurate reaction rate coefficients for the key chemical reactions. Such reaction rate measurements must accurately measure low concentrations of reactants and products as functions of time. For most chemical species, optical absorption can measure concentrations very selectively. However, the sensitivity of conventional optical absorption detection is often insufficient to allow reaction rate measurements.

Approach: We are developing cavity ring-down (CRD) optical absorbance measurement technology to meet the requirements imposed by chemical kinetics experiments in gas and condensed phases.

Results: This year we have applied resources to two activities: 1) experiments that have validated the usefulness of CRD detection for reaction rate measurements in the gas-phase, and 2) theory projects that have enabled us to invent optical devices capable of measuring reaction rates in liquids and thin films using CRD detection.

No previous studies have demonstrated that CRD detection methods can measure reaction rates accurately. To confirm that CRD detection is useful, we examined the reactions:

$$CH_3CH_2\cdot + CH_3CH_2\cdot \xrightarrow{k_1} products$$

$$CH_3CH_2\cdot + O_2 + Ar \xrightarrow{k_2} CH_3CH_2OO\cdot$$

$$CH_3CH_2OO\cdot + CH_3CH_2OO\cdot \xrightarrow{k_3} products$$

in a slow-flow flash photolysis reactor. Ethyl radicals were detected with 220 nm laser light and ethylperoxy radicals were detected with 270 nm laser light. Using CRD detection to measure the time-dependent concentrations of these radicals, our experiments reproduced the accepted room-temperature reaction rates of all three reactions. Additional experiments demonstrated that CRD detection has excellent response linearity, sensitivity, and operational utility within a flash-photolysis reactor.

Current optical designs used in CRD detectors cannot be productively applied to species detection in liquids. Solutions can pollute or damage the mirrors, and the substantial refractive indices of liquids make most designs unfeasible. We have invented optical devices that allow CRD concentration measurements in liquids and thin films. Most of these designs are based upon monolithic cavities constructed from polygons of optical material. Although conceptually simple, each device incorporates subtle optical features that were discovered and optimized through exhaustive computational modeling of wave propagation. These optical models required the full capabilities of the NIST Cray supercomputer.

Future Plans: In FY 1997, we will use cavity ring-down spectroscopy to determine rates of reactions that involve ethyl, methyl, peroxy, and propargyl radicals. We anticipate that the improved sensitivity obtained with CRD detection will also enable us to obtain accurate rates for bimolecular reactions between two *different* free radicals. Radical-radical cross-reaction rates are extremely difficult to obtain because the experiments must measure the temporal decay of the *absolute* concentrations of two free radicals simultaneously. Such experiments will incorporate two lasers, two CRD detectors, and enhanced computer software. If successful, they should allow explorations of sparsely studied but important topics in chemical kinetics.

Using the novel optical devices designed during the past year, we will also extend our studies of chemical reaction rates into the liquid phase. We will test these novel devices for linear response, sensitivity, and selectivity for liquid-phase species. We will also validate their use by measuring the rates of well-known photolytically-induced liquid-phase reactions.

Publications:

Pipino, A.C.R., Hudgens, J.W., and Huie, R.E., "***Condensed Matter Ring-Down Spectroscopy with a Total-Internal-Reflection Minicavity,***" (in press).

Atkinson, D.B. and Hudgens, J.W., "***Chemical Kinetic Studies Using Ultraviolet Cavity Ring-Down Spectroscopic Detection: Self-Reaction Rates of Ethyl and Ethyl-peroxy Radicals and the Reaction Rate of O_2 +C_2H_5 ---> $C_2H_5O_2$,***" (in press).

9. *Measuring and Modeling of Solute-Solvent Interactions in Supercritical Fluids*

T.J. Bruno and A.F. Lagalante

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

Problem: As a result of the Montreal Protocol and subsequent international treaties, many industrially important chlorinated solvents used in cleaning and extraction processes will become obsolete. Replacements for the chlorinated solvents are likely to come from fully or partially fluorinated alkanes, ethers, or ketones that possess negligible ozone-depletion potential. Unlike their chlorinated analogs, many of the fluorinated alternative solvents are gases under ambient conditions, and their thermophysical properties make them promising for both conventional liquid extraction and for near-critical and supercritical fluid extraction. The most important piece of thermophysical properties data to assess the feasibility of an extraction process is the solute-solvent phase equilibrium. Despite some success in treating simple binary systems, serious limitations exist in equation-of-state modeling approaches that use only physical properties of the solute and solvent to model supercritical and near-critical solute solubility. This is due to the difficulty of accounting for the often disparate chemical natures of the solute and solvent.

Approach: A given solution process using chlorinated solvents is modeled empirically as the dependent variable in a multivariate statistical analysis. The independent variables to the multivariate statistical model include quantitative measurements of solute-solvent interactions and additional state dependent terms. Solute-solvent interactions are quantified using the Kamlet-Taft solvatochromic parameters. These parameters are empirically determined values of three solvent-solute interactions: acidity, basicity, and dipolarity-polarizability. Work is in progress to extend the Kamlet-Taft solvatochromic chemical parameters to alternative solvents in the subcritical and supercritical phases. The results of the model will aid in the replacement of chlorinated solvents through prediction of the solubility of industrially relevant compounds in alternative solvents. The model will provide insight into our understanding of the solution process itself.

Results and Future Plans: In recent years, we have designed and constructed numerous instruments for the quantification of solubilities of solutes in subcritical and supercritical fluids. These include both dynamic methods and static-equilibrium-spectroscopic measurements. Solutes studied have ranged from classes of organometallics (such as metal β-diketonates and calixarenes) to physiologically active natural products (such as β-carotene and capsaicin). In the near future we plan on acquiring and modifying a commercially available supercritical fluid extractor to measure not only solid-fluid equilibrium using the alternative solvent, but also the extraction potential of the alternative solvents in naturally occurring matrices.

The Kamlet-Taft parameters for fluorinated ethane-based alternative solvents have been measured using the high-pressure equilibrium cells constructed in our laboratory. Results show a constant basicity and acidity value over the gas to liquid density range of the fluorinated ethanes, whereas the dipolarity-polarizability of the solvent can be fit to a third-order poly-nomial in the pure fluid density. For the fluorinated ethanes, this parameter increases in a stepwise manner with increasing unsymmetrical fluorine substitution about the ethane molecule. The solubilities, as predicted from the multivariate statistical model, will be compared to the experimentally measured solubilities of specific solutes in existing and new solubility measurement instrumentation. A predictive solubility model will suggest suitable alternative solvents and extraction conditions to substitute for an obsolete chlorinated solvent. Although initially the model will be tested using the pure fluorinated alternative solvents, it is envisioned that the model will be applicable to other classes of solvents and to zeotropic and azeotropic mixtures of solvents. Other types of solution equilibria will be modeled including chromatographic retention data and rate constants of reactions in the alternative refrigerant solvents.

Publications:

Lagalante, A.F., Jacobson, R.J., and Bruno, T.J., ***"UV/VIS Spectroscopic Evaluation of 4-Nitropyridine N-Oxide as a Solvato-chromic Indicator for the Hydrogen-Bond Donor Ability of Solvents,"*** J. Org. Chem. 61, 6404-6406 (1996).

10. Measurements and Database for Adsorptive Separations

*T.J. **Bruno**, J.E. Mayrath, H.J.M. Hanley, F. Tsvetkov (Solar Dynamics, Inc., Israel), and A. Lewandowska (Univ. Colorado)*

Objective: To provide basic data to facilitate the design of efficient and effective adsorptive separations for process and environmental applications.

Problem: Adsorptive separation techniques represent a versatile and selective family of methods that is especially applicable to dilute solutions. This is a concentration regime that is particularly problematical and costly for many industries, including chemical processing, energy, pharmaceuticals, and almost all phases of environmental remediation. As currently practiced, the design of adsorptive separations relies greatly on intuition. Adsorbents are customarily characterized in terms of surface area and pore size distributions, which are concepts that do not adequately account for the actual structures and attractive potentials that increase the concentrations of adsorbates on the surfaces of adsorbents. Moreover, thermodynamic properties (such as isotherms and enthalpies) measured on "standard adsorbents" cannot fully account for porosity and adsorptive capacities for other adsorbates, nor can such measurements account for properties at conditions differing from those of the standard measurements. This situation makes the rational design of adsorptive separations nearly impossible.

Approach: This project consists of a data compilation effort and an experimental measurement and modeling effort. The data compilation work encompasses a literature review and critical evaluation of the literature focused on theory and measurements concerning adsorption from the gas phase on carbon, zeolites, and novel materials (polymers, urea, clathrates, etc.). Other physical property measurements of the adsorbents are correlated (where possible) with the customary or classical adsorption isotherms and areas and porosities. The current experimental effort concerns the enthalpy of adsorption of pollutants on unmodified clays (and clays modified with surfactants), and also the measurement of the skeletal density of adsorbents. We are using a chromatographic approach to measure the enthalpies, in which the clays (laponite, bentonite, montmorillonite, etc.) are deposited on glass spheres which are then packed into columns. The temperature dependence of the specific retention volume provides the enthalphic information. The skeletal density apparatus, which has just been completed, consists of a microbalance enclosed in a vacuum chamber. Necessary gas manifolds allow the introduction of helium and other gases at known temperatures and pressures, and therefore, densities. One then measures the mass at several gas densities at several sample temperatures, yielding the adsorbent skeletal density.

Results and Future Plans: The results of the comprehensive literature search have been compiled into a database that is searchable by a variety of keyword approaches. The results of the literature review have revealed that there are no recognized or *de facto* standard adsorbents suitable for characterization. Adsorbent properties are known to vary from batch to batch, making correlations of measurements between batches highly uncertain. Data on a variety of adsorbates that can be traced to specific material lots or batches are scarce. Moreover, regeneration appears to change adsorbent characteristics, and few studies of characterization and physical properties as a function of adsorbent history are available. Surface area and pore size distributions were found to be virtually meaningless for materials which are thought to provide material separation on the basis of size and/or shape.

Enthalpy of adsorption measurements have been completed for a large number of hydrocarbons and pollutant species on laponite clay. These data provide an insight into the relative strength of the adhesion of such pollutants on these common adsorbents. Correlations have emerged that relate structural features such as carbon number to the measured enthalpy.

11. Measurements and Database for Membrane Separations

*J.J. **Pellegrino**, M.R. Yetzbacher, J. Tighe, B.R. Mattes (Los Alamos National Laboratory), and Michael Guiver (National Research Council of Canada)*

Objectives: To develop improved quantitative structure/property prediction methods for polymeric membrane materials based on high-quality measurements of sorption and transport of gases and vapors in well-characterized systems; to compile property data on industrially-significant materials used for membrane-based separations.

Problem: Although polymeric materials are used in membrane and adsorptive separation processes, a significant barrier to the development of new materials and optimum use of existing materials is the lack of *a priori* predictive capabilities for the transport properties of mixtures in any selected polymer.

Approach: This program has a measurements and modeling component and a data compilation component. Measurements of gas and vapor sorption and diffusion in polymers are critical for development of techniques to predict transport properties. These measurements provide a means to compile the effects of both chemical and structural subgroups in the polymer, and ultimately to delineate rational design criteria for separations. Through our collaborations with Los Alamos National Laboratory and the National Research Council of Canada, we have access to materials whose chemistries can be varied in well-defined ways. In addition, the polymers under study (polysulfone, polyperfluorosulfonic acid, and polyaniline) represent both commercial and cutting-edge materials. Improved processes for obtaining high-purity oxygen and nitrogen from air, upgrading of natural gas, recovery of hydrogen from refinery streams, recovery and purification of olefin streams, and water purification are examples of important industrial uses of membranes. The data compilation component of this research program, which has just begun, consists of the development of an Internet-accessible database of polymeric material properties that are important for membrane separation design.

Results and Future Plans: Four sorption systems based on pressure decay methods are currently in operation. During the past year, data have been obtained for determining the film density and sorption and diffusion coefficient of argon, nitrogen, and oxygen in polyaniline films doped with HF and HCl, and in the unmodified, polysulfone base polymer. A detailed sensitivity analysis of the uncertainty associated with pressure-decay methods for measuring gas sorption has been completed to provide guidance in designing and operating such an apparatus. Also, refurbishment and re-calibration of the multicomponent gas flow apparatus for measuring transport of gas and vapor mixtures through membranes has been nearly completed. Work has begun on constructing a membrane technology database using a query-based search engine accessed via World Wide Web browser programs. A variety of software tools have been created and tested. This work addresses 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 interaction properties include permeability, solubility, diffusion, activation energies, and interaction parameters of feed materials with the membrane materials. The membrane properties include transition temperatures of membrane polymers, densities, elastic moduli, etc. During the next year literature data will be compiled and incorporated in the database.

Publications:

Pellegrino, J.J., Radebaugh, R., and Mattes, B.R., "***Gas Sorption in Polyaniline. 1. Emeraldine Base,***" Macromolecules 29, 4985-4991 (1996).

12. *Database for Neutral/Ion Kinetics for Plasma Processes*

L.W. Sieck, J.T. Herron (Contractor), D.S. Green (836), and T.J. Buckley

Objective: To develop a laboratory-validated database for modeling the processes associated with non-thermal plasma treatment of gas streams.

Problem: Advances in effective manufacturing and environmental cleanup can be achieved by exploiting non-thermal plasma treatment. In such discharges, chemical change is driven by a variety of processes including ionization, molecular excitation, ion-electron recombination, fragmentation of ions and excited molecules, and reactions of radicals, atoms, and ions. The quantitative modeling of these processes requires a reliable database on the kinetics of the elementary reactions involved.

Approach: The program has centered around the creation and validation of an ion chemistry database for use in modeling plasma processes. In addition, a neutral-chemistry component will be added to represent more completely the total chemical processes. The creation of the ion chemistry database involves data collection and evaluation, measurement of specific rate parameters, and the verification of the model with the NIST high-pressure mass spectrometer.

Results and Future Plans: Present efforts are focused mainly on the removal of volatile organic compounds and nitrogen oxides from humid air streams typical of jet-engine test beds and from diesel sources. The first task was to survey and assess the available literature and create a recommended database for the reactions of both positive and negative ions arising from the irradiation of both wet and dry air under the conditions pertinent to measurements in the NIST high-pressure mass spectrometer. These evaluations included both second- and third-order rate parameters and the temperature dependencies of the reactions. Next, modeling was performed with the recommended database to generate predicted temporal profiles of the positive ions in wet or dry air under the conditions to be employed in the laboratory experiments. Then, positive ions were created in the NIST high-pressure mass spectrometer, and their temporal profiles were monitored. These measurements provided information on the yields, mechanisms, and parent-daughter relationships in these systems and, very importantly, on the terminal ion distributions. Measurements were carried out over a very wide temperature range, 300 K to 700 K, with 400 pa to 533 pa (3 torr to 4 torr) total pressure. This has allowed us to validate the basic chemical model which will be employed to simulate non-thermal plasma reactors. These results have been quite successful, supporting the validity of the model being employed.

The available literature pertinent to positive ion chemistry in both wet and dry air at nominally atmospheric pressure was surveyed and assessed. A recommended database was created for the temperature range 300 K to 500 K. The database includes pressure effects and the conversion from third- to second-order kinetics, which is of particular importance when attempting to model atmospheric reactions. The database also includes switching reactions involving association and the formation of cluster ions, etc. The goal of this activity is to develop a reliable predictive scheme for atmospheric-pressure reactors. This predictive scheme is based on the lower-pressure scheme, which has been validated by comparison with results from the NIST high-pressure mass spectrometer. In addition to the studies involving wet and dry air alone, experiments were carried out with the addition of a few µg/g of toluene as a representative hydrocarbon with a low ionization potential, or of carbon tetrachloride as a molecule that undergoes dissociative electron attachment. These experiments demonstrated the dramatic effect these additives have on the air system, including an apparently complete quenching of the NO_x ion chemistry. The study of the air/toluene system has been completed, including the creation of an evaluated database and modeling of the system to validate the mechanism. Only preliminary experiments have been performed on the air/CCl_4 system.

In the coming year, we will create a compact chemical kinetics database for ion chemistry at one atmosphere incorporating key reactions representative of volatile organic compounds which influence the destruction of nitrogen oxides during non-thermal plasma treatment. A generic recommended database for negative ion kinetics in humid air at atmospheric pressure will also be assembled. Particular attention will be placed on the role of O^-, since formation of O_2^- and its subsequent influence on the anionic mechanism appear to be minimal under high-field conditions. Laboratory validation of the competitive ion kinetics and important parent-daughter relationships will be conducted as required. Due to the complexity of the mechanisms, it is eminently clear that predictive models for non-thermal plasma applications must be improved, requiring extensive and sophisticated laboratory validation. Therefore, we are upgrading the NIST high-pressure mass spectrometer by combining the unique components of the outdated pulsed high-pressure mass spectrometer with triple-quadrupole electronics and a vacuum chamber presently on site to produce a hybrid measurement facility. To this will be added modern data acquisition and handling capabilities, and more reliable pumping, leading to a world-class instrument.

Publications:

Green, D.S., Herron, J.T., and Sieck, L.W., "***Pulsed Electron Beam and Electric Discharge Processing of Volatile Organic Compounds in Humid Air,***" Proc. Second International EPRI (Electric Power Research Institute) Symposium on Advanced Oxidation Technology, San Francisco, CA (1996).

13. *Radiolytic and Photolytic Studies for Advanced Oxidation Technologies*

P. Neta, R.E. Huie, Z.B. Alfassi (Ben Gurion Univ., Israel), and M. Al-Sheikhly and J. Silverman *(Univ. Maryland)*

Objective: To determine the kinetics and mechanisms of the basic reactions underlying various advanced oxidation technologies in the liquid phase and, in collaboration with outside researchers, to develop novel approaches to the destruction of hazardous compounds in waste water.

Problem: Advanced oxidation technologies are all based on the generation of reactive free radicals and their subsequent reactions with recalcitrant pollutants. By developing a basic understanding of these processes, we can provide engineers with the information needed to choose among the competing systems, develop new approaches, or to optimize any particular process. By use of the pulse radiolytic and flash photolytic capabilities at NIST, the basic data required can be generated as part of a larger program to provide evaluated kinetic data to the Advanced Oxidation community. In addition, NIST possesses expertise and facilities which can be used in the development of new processes based on radiolysis, and these studies can combine synergistically with the investigations of the basic chemical processes.

Approach: Experiments to elucidate the individual steps involved in advanced oxidation processes are carried out by use of pulse radiolysis or flash photolysis techniques for the rapid generation of the free radical intermediates which are subsequently detected by optical methods. The development of new processes has involved the use of gamma radiolysis with detection of chemical changes by use of ion chromatography and gas chromatography/mass spectrometry. An important component of this effort has been the development of data compilations which will serve as the basis for the creation of a database for modeling advanced oxidation processes.

Results and Future Plans: Studies of the kinetics and mechanisms of the reactions of organic peroxyl radicals, which are key intermediates in any advanced oxidation process, have been continued and extended to the interactions of these radicals with transition-metal ions, which are often used as catalysts. In the past, we have examined the reactions of primary radicals formed in the radiolysis of some solvents with several groups of important compounds, such as phenols and halogenated hydrocarbons, whose destruction is an environmental challenge.

During the past year, we studied two groups of compounds: trichloro- and trifluoroethanol and related compounds; and polychlorinated biphenyls (PCBs) and related compounds. Studies of the first group have included fundamental investigations of their reactivity toward primary radicals arising from water radiolysis (e.g., hydroxyl radicals and solvated electrons) and the subsequent reactions of the derived radicals. The work on PCBs involved the radiolytic destruction of selected compounds under different conditions and the elucidation of the mechanisms involved in these processes. During the past year, we studied the radiolytic destruction of dichlorobiphenyls in aqueous alcohol solutions and began to study tetrachloro- and decachloro-biphenyls in various solvent mixtures. This work will continue in FY 1997.

Publications:

Alfassi, Z.B., Khaikin, G.I., Johnson III, R.D., and Neta, P., ***"Formylmethyl and Formylmethylperoxyl Radicals and Their Chlorinated Derivatives. Formation and Reactions in Irradiated Aqueous Solutions,"*** J. Phys. Chem. 100, 15961 (1996).

Khaikin, G.I., Alfassi, Z.B., and Neta, P., ***"Inter- and Intramolecular Redox Reactions of Substituted Phenylperoxyl Radicals in Aqueous Solutions,"*** J. Phys. Chem. 99, 16722 (1995).

Neta, P., Grodkowski, J., and Ross, A.B., ***"Rate Constants for Reactions of Aliphatic Carbon-Centered Radicals in Aqueous Solution,"*** J. Phys. Chem. Ref. Data 25, 709 (1996).

14. Thermophysical Properties of Alternative Refrigerants

M.O. McLinden, D.R. Defibaugh, K.A. Gillis, C.D. Holcomb, M.L. Huber, J.W. Hurly, A.R. Laesecke, E.W. Lemmon, J.W. Magee, M.R. Moldover, S.L. Outcalt, R.A. Perkins, and L.A. Weber (Contractor)

Objective: To provide industry with high-accuracy property data and models for alternative refrigerants and refrigerant mixtures.

Problem: The chlorofluorocarbon (CFC) and hydrochlorofluorocarbon (HCFC) refrigerants must be replaced with fluids that do not deplete stratospheric ozone. To evaluate the energy efficiency, capacity, etc. of any fluid in a thermodynamic cycle, or to design equipment using a new fluid, knowledge of the thermophysical properties is required.

Approach: Our work on alternative refrigerants represents a comprehensive approach to a major problem and has been supported by multiple government agencies and industry groups. The Division's work in this area includes experimental measurements at several levels of detail and the compilation and evaluation of literature data. These data provide the input information for developing the models which form the basis of the REFPROP database (see Report 15). Also included are technology transfer activities and leadership and participation in international collaborations.

Results and Future Plans: While suitable pure-fluid replacements have been found for the CFCs, the requirements of most HCFC applications require a blend of components. Thus, the work on alternative refrigerants is shifting increasingly toward mixtures. Measurements of the vapor-liquid equilibria (VLE), including bubble-point pressures and coexisting liquid and vapor compositions and densities for nine binary and ternary refrigerant mixtures have been completed. These systems cover a wide range of fluid behaviors, including azeotropes and liquid-liquid immiscibility. Comprehensive measurements, including studies of pressure-volume-temperature behavior, heat capacity, and VLE, have been completed for R125/143a and R41/CO_2 mixtures. Measurements of viscosity and thermal conductivity are underway for five binary refrigerant systems. These measurements will be used to evaluate the accuracy of existing mixture models and to define future needs for the transport properties of mixtures.

Work continues on pure refrigerants. The U.S. Navy is retrofitting its fleet with refrigeration plants using R236fa. The data required for the retrofit were developed in the Division, as were the data for other candidate refrigerants that the Navy considered and rejected. Two new fluids, R41 (fluoromethane) and trifluoroiodomethane, are being investigated. R41, alone or in a blend with carbon dioxide, is a candidate for low-temperature applications currently using CFC-13; thermodynamic measurements and an equation of state have been completed. Blends of trifluoroiodomethane and one or more HFCs have been suggested as a retrofit fluid for servicing existing equipment using CFC-12. Measurements on this fluid and its blends are underway.

In the international arena, NIST coordinated an evaluation of equations of state for R32 and R125 by Annex 18 of the International Energy Agency; this process led to the adoption of international standard formulations for these fluids. We have completed a comprehensive formulation for the viscosity of R134a under the auspices of the IUPAC Subcommittee on Transport Properties. This work, which will lead to an international standard, included an extensive evaluation of literature data and new measurements to fill in gaps or resolve differences in the existing data. A similar task for thermal conductivity is underway.

The work on alternative refrigerants will wind down in the coming years as industry settles on replacement fluids for various applications, but we expect continued work at a reduced level of effort for many years to come.

Publications:

Defibaugh, D.R. and Moldover, M.R., "***Compressed and Saturated Liquid Densities for 18 Halogenated Organic Compounds,***" J. Chem. Eng. Data (in press).

Gillis, K.A., "***Thermodynamic Properties of Seven Gaseous Halogenated Hydrocarbons from Acoustic Measurements:*** $CHClFCF_3$, CHF_2CF_3, CF_3CH_3, CHF_2CH_3, $CF_3CHFCHF_2$, $CF_3CH_2CF_3$, ***and***

***$CHF_2CF_2CH_2F$*,"** Int. J. Thermophysics 18, 73-136 (1996).

Laesecke, A., Perkins, R.A., and Howley, J.B., ***"An Improved Correlation for the Thermal Conductivity of HCFC123 (2,2-dichloro-1,1,1-trifluoroethane),"*** Int. J. Refrigeration 19, 231-238 (1996).

Magee, J.W., ***"Isochoric p-ρ-T Measurements on Difluoromethane (R32) from 142 to 396 K and Pentafluoroethane (R125) from 178 to 398 K at Pressures to 35 MPa,"*** Int. J. Thermophysics 17, 803-822 (1996).

Schmidt, J.W., Carrillo-Nava, E., and Moldover, M.R., ***"Partially Halogenated Hydrocarbons $CHFCl$-CF_3, CF_3-CH_3, CF_3-CHF-CHF_2, CF_3-CH_2-CF_3, CHF_2-CF_2-CH_2F, CF_3-CH_2-CHF_2, CF_3-O-CHF_2: Critical Temperature, Refractive Indices, Surface Tension and Estimates of Liquid, Vapor and Critical Densities,"*** Fluid Phase Equilibria 122, 187-206 (1996).

Weber, L.A. and Silva, A.M., ***"Design of a High-Pressure Ebulliometer, with Vapor-Liquid Equilibrium Results for the Systems $CHF_2Cl + CF_3$-CH_3 and CF_3-$CH_2F + CH_2F_2$,"*** Int. J. Thermophysics, 17, 873-888 (1996).

15. Reformulation of the NIST Thermodynamic and Transport Properties of Refrigerants and Refrigerant Mixtures Database (REFPROP)

M.O. McLinden, S.A. Klein (Univ. Wisconsin), E.W. Lemmon, and A.P. Peskin

Objective: To make the best equation-of-state and transport-property models available to designers and manufacturers of refrigeration machinery in the most useful forms, i.e, as well-documented Fortran subroutines and with a modern, Windows-based graphical user interface.

Problem: While the REFPROP database has seen widespread acceptance for the property calculations necessary to evaluate new refrigerants, it suffers from an awkward user interface and a structure that makes the addition of new models difficult.

Approach: We have completely rewritten REFPROP combining Fortran-based subroutines implementing the fluid property models with a modern graphical user interface (GUI) written in Pascal. The GUI accesses the Fortran routines via a dynamic link library. Fluid-specific coefficients are stored in text files (with one file per fluid) allowing fluids to be added or deleted without recompiling the entire database.

Results and Future Plans: The Fortran property subroutines implement three models for the thermodynamic properties of pure fluids. Comprehensive, high-accuracy Helmholtz or modified Bennedict-Webb-Rubin (MBWR) equations of state are used whenever available; these models (which currently number about 20) include ones taken from the literature as well as those developed at NIST. An extended corresponding states (ECS) model is used for fluids with limited data. Mixture thermodynamic properties are calculated using a new model which applies mixing rules to the Helmholtz energy of the pure components. The transport properties are modeled with pure-fluid correlations or a new, enhanced ECS model. The property routines are highly modular; the GUI, or anyone using the subroutines directly, would call only high-level, model-independent routines which, in turn, call "core" routines implementing the various models. Such a structure is designed to simplify the addition of future models.

The new graphical user interface provides a convenient means to calculate and display properties. It provides easy access to numerous options controlling the units, reference state, and properties to be displayed. Computed properties are displayed in spreadsheet-style scrollable windows. Multiple windows are allowed and results can be copied and pasted to other applications. Information screens document the models, including literature references. Plotting capabilities and an on-line help system are also included.

The database is currently undergoing testing by NIST staff and several industrial users. A public release date in FY 1997 is on schedule. The modular structure of the new code makes it easily adaptable to other NIST databases, including the NIST Thermophysical Properties of Pure Fluids Database (NIST Standard Reference Database 12) and the NIST Mixture Property Program Database (NIST Standard Reference

Database 14), and these will be put into the new structure.

Publications:

McLinden, M.O. and Klein, S.A., "*A Next Generation Refrigerant Properties Database*," in Proc. 6th International Refrigeration Conference, Purdue, West Lafayette, IN, 409-414 (1996).

Klein, S.A., McLinden, M.O., and Laesecke, A., "*An Improved Extended Corresponding States Method for Estimation of Viscosity of Pure Refrigerants and Mixtures*," Int. J. Refrigeration (in press).

16. The NIST/ASME Thermophysical Properties of Water Database (STEAM)

A.H. Harvey, S.A. Klein (Univ. Wisconsin), A.P. Peskin, E.W. Lemmon, D.G. Friend, J.M.H. Levelt Sengers, C.J. Wardell (Univ. Colorado), and M.A. Killeen (Univ. Colorado)

Objective: To develop and disseminate standard reference data for the thermophysical properties of water over wide ranges of conditions.

Problem: Water is ubiquitous in industry and science as a calibration fluid, solvent, working fluid, etc., and its properties have been studied extensively for many years. The standard thermodynamic formulation, which has been widely accepted and distributed through the NIST Standard Reference Data Program (SRDP) since the mid-1980s, has been shown to suffer from several deficiencies. In particular, the behavior of the thermodynamic surface in the critical region may not be sufficiently accurate, the metastable regions are not well described, the extrapolation behavior is not sufficiently well-behaved to allow calculation in some important regions, and perhaps most notably, its formulation was based on the International Practical Temperature Scale of 1968 which has been superseded by the International Temperature Scale of 1990. Because of the many applications and independent standards which are based on the properties of pure water, it is important that NIST continue to provide the best widely accepted formulations as they are developed.

Approach: Through many years, we have been actively involved in the development efforts, testing, and deliberations of the International Association for the Properties of Water and Steam (IAPWS), which is an international body with participation by many of the major industrial users of water and steam as working fluids. In late 1995, IAPWS adopted a new formulation for thermodynamic properties, and through its association with the U.S. National Committee for IAPWS [located within a research committee of the American Society of Mechanical Engineers (ASME)], NIST was asked to provide the National implementation of this standard. In addition, these changes and new experimental data have necessitated revisions of related property standards, such as the transport properties and the dielectric constant of water and steam. We have been working with other colleagues in the areas of bibliographic work, data evaluation, and development of theoretically based correlations for the development of updated standards in these areas.

Results and Future Plans: Work on the NIST/ASME Thermophysical Properties of Water Database (Version 2.0) has been completed and is undergoing final review prior to its release by the SRDP. This is a Windows-based program that fully implements the new IAPWS formulation for thermodynamic properties and allows calculations of other properties based on interim equations; its graphical user interface allows the user to generate tables and plots and to transfer information to or from other computer programs. In addition, the new dielectric constant formulation was completed in FY 1996, and interim transport property formulations have been implemented in the computer code. Work is continuing on improvements in the transport properties, an update to the NIST/ASME Steam program to incorporate a revised refractive index formulation and additional properties related to Debye-Hückel coefficients, and cooperation with the development of a separate implementation geared toward very fast computer codes for specialized industrial uses.

Publications:

Klein, S.A. and Harvey, A.H., "*NIST/ASME Steam Properties,*" NIST Standard Reference Database 10, Version 2.0; National Institute of Standards and Technology, Standard Reference Data Program, Gaithersburg, MD (1996).

Fernández, D.P., Goodwin, A.R.H., Lemmon, E.W., Levelt Sengers, J.M.H., and Williams, R.C., "***A Formulation for the Static Permittivity of Water and Steam at Temperatures from 238 to 873 K at Pressures up to 1200 MPa, Including Derivatives and Debye-Hückel Coefficients***," J. Phys. Chem. Ref. Data (in press).

17. Dilute Impurities in Fluid Systems

A.H. Harvey, J.C. Bellows (Westinghouse Power Generation), M.E. Mullins (Michigan Tech. Univ.), A.P. Peskin, G.R. Hardin, J.C. Rainwater, M.L. Huber, and C.J. Wardell (Univ. Colorado)

Objective: To develop models and evaluate data that can be used to describe the solubility of dilute solutes and impurities in fluid systems and the processes of dissolution and precipitation of solid-phase species in aqueous systems.

Problem: The problem of phase partitioning in fluid systems is a major concern in a variety of industrial applications. These include the control of environmental pollutants, quality control issues in products and working fluids, avoidance of deposition within pipelines or other channels, and stimulation of deposition to optimize surfaces or catalyze surface chemical reactions. We have sought to address several issues within this broad area, but we will concentrate here on steam quality issues in an advanced turbine design project. Proposed combustion turbine designs will use steam cooling, with small cooling channels running through vital parts. The temperature gradients within these systems, as well as the flow patterns, imply the possibility that impurities dissolved in any portion of a closed cycle will deposit within and ultimately block the cooling channels. This could lead to catastrophic failure. Unfortunately, adequate data for impurity species known to present problems do not exist in the region of main concern with temperatures from 600 K to 900 K and pressures between 2 MPa and 4 MPa.

Approach: Solution of the most immediate problem outlined above required an extensive bibliographic search and study of the available data and models. The systems of interest included the aqueous solutions of sodium chloride, sodium hydroxide, sodium sulfate, sodium phosphate, iron oxides, copper oxides, and silicon dioxide. The conditions of interest correspond to low steam densities (of the order of 0.5 mol/L to 1 mol/L) where few reliable data exist. Two classes of models were considered: a physical model based on the fugacity of the solute and a fugacity coefficient for the vapor phase; and a reaction model, where the solubility is considered as a complexation between a number of solvent (water) molecules and a solute molecule.

Results and Future Plans: The available data for these systems tended to be both inconsistent and insufficient to achieve a firm correlation of the solubility behavior; the available models are also not adequate to make reliable predictions. In part, the problem stems from the very wide variation in solubility in the range of interest. For some of the species, we have established estimates that can be used to bound the behavior of the true solubility. The work has enabled the turbine designers to determine water quality specifications which should ensure that deposition is not a severe problem in the proposed cooling channel configuration. Related research during FY 1996 focused on the following topics: the temperature dependence of the Henry's law constant, improvements in our estimates of vapor pressures for complex molecules, the suitability of virial expansions for describing solubility, the deposition of chemically reacting solid precipitates in a complex flow field, and modifications to an extended corresponding states model to account for complex, heavy solutes. Additional studies of the Henry's law constant and of precipitation in other complex systems are underway or planned.

Publications:

Harvey, A.H. and Bellows, J.C., "***Evaluation and Correlation of Steam Solubility Data for Salts and Minerals of Interest in the Power Industry***," Report to Sponsor, 1996 and NIST Technical Note (in press).

Harvey, A.H., "***On the Suitability of the Virial Equation for Modeling the Solubility of Solids in Supercritical Fluids***," Fluid Phase Equil. (in press).

18. *Ammonia-Water Mixtures for Use in Power Cycles*

D.G. Friend, J.C. Rainwater, M.L. Huber, A.L. Olson (Univ. Colorado), A. Nowarski (Univ. Cracow, Poland), R. Tillner-Roth (Univ. Hannover, Germany), C.D. Holcomb, J.W. Magee, R.A. Perkins, T. Lüddecke (Univ. Hannover, Germany), and S. Kiselev (Inst. Oil and Gas Res., Russia)

Objective: To establish standard reference thermophysical property surfaces for mixtures of ammonia and water over a broad range of temperature, pressure, and composition.

Problem: Improvements in the efficiency of power generation plants are often made in the areas of heat exchangers, combustion engineering, turbine design, more extreme operating conditions, and development of combined cycle operations. Currently, plants operate with steam in a standard Rankine cycle, and recent improvements may be considered marginal. Drastically redesigned systems have been proposed, however, that use the binary ammonia-water system as a working fluid, and increases in efficiency of several percent have been predicted for some applications. Several major industrial users have obtained license agreements to implement this approach. Critical design decisions and corporate risk analysis processes require property parameters which are not currently available. In addition to these engineering and other practical concerns, problems in the optimization of these new cycles and in the development of performance warranty protocols have not been resolved because of uncertainties in the properties of the working fluid. The major interest is in phase boundaries, density, enthalpy change, surface tension, thermal conductivity, and viscosity, although the full thermodynamic surface is required to develop cycle parameters. This binary system is also the working fluid in some refrigeration applications, including prospective domestic heat pump and air conditioning technologies; the optimization of designs for these systems also requires reliable standard property information.

Approach: The project requires an extensive effort of bibliographic study and evaluation of existing data. Experimental measurements will be obtained for selected properties at the most important state points, including measurements of vapor-liquid equilibrium (VLE), density, isochoric heat capacity, thermal conductivity, and viscosity. Some apparatus modification and development is required to perform the measurements. The modeling effort will include studies based on scaling theory to describe the high-pressure region of the phase boundaries, as well as several versions of an extended corresponding states (ECS) algorithm; additionally, a two-fluid Helmholtz energy model is being developed to describe the thermodynamic surface of the mixture.

Results and Future Plans: A workshop on the thermophysical properties of ammonia-water mixtures, geared to industrial users of this information, was held during the past year. Efforts continued in the areas of data collection, evaluation, and organization. The initial version of a two-fluid Helmholtz energy model has been optimized to the available data, and comparisons with these data have allowed clear evaluation of the data situation. The scaling theory approach was used to generate densities for the general thermodynamic surface, and this procedure was iterated to establish an accurate self-consistent formulation. The ECS model now incorporates state-dependent shape factors and promises to allow extrapolation into the high temperature regions of major interest.

Measurements have been completed for the VLE surface of the mixture in the region accessible to the current apparatus. Acquired data include phase boundary pressures, compositions, and densities. Measurements of the isochoric heat capacity for ammonia-rich mixtures have begun with temperatures up to 523 K. Work will continue on calorimetry, thermal conductivity measurements, completion of a new viscometer for ammonia-water measurements, and additional data acquisition, depending on the requirements of the sponsors. The thermodynamic surfaces will be re-optimized based on these new data, a user interface will be developed, and descriptions of the transport property surfaces will be studied.

Publications:

Tillner-Roth, R. and Friend, D.G., ***"Survey and Assessment of Available Measurements of Thermodynamic Properties of the Mixture (Water + Ammonia),"*** J. Phys. Chem. Ref. Data (in press).

Tillner-Roth, R. and Friend, D.G., ***"A Helmholtz Free Energy Formulation of the Thermodynamic***

Properties of the Mixture (Water + Ammonia)," J. Phys. Chem. Ref. Data (in press).

Friend, D.G. and Haynes, W.M., "***Report on the Workshop on the Thermophysical Properties of Ammonia + Water Mixtures,***" NIST Tech. Note (in press).

19. Environmental Fate of Industrial Compounds

R.E. Huie, V.L. Orkin (Institute of Energy Problems of Chemical Physics, Russia), D.G. Archer, B. Laszlo (Central Research Institute for Chemistry, Hungary), A. Fahr, T.J. Buckley, and M.J. Kurylo

Objective: To provide the basic kinetic and thermodynamic data needed to understand and determine the fate of industrial compounds in the atmospheric and hydrologic environments.

Problem: Chlorofluorocarbons and halons have served many of the Nation's needs as solvents, propellants, refrigerants, fire suppressants, etc. The use of these compounds is being phased out due to their adverse effect on the stratospheric ozone layer. This phaseout must occur with limited disruption of the various production and service industries that depend on such chemicals. For many uses, completely satisfactory replacements have not yet been identified. An important example of this is fire suppression. Industry is now beginning to look beyond the halogenated organic compounds to new classes of chemicals for which, typically, there is little environmental fate information. To ensure an orderly transition to such new chemicals, we are assisting industry in evaluating their environmental chemistries.

Data on the thermodynamic properties of materials released into the environment are also required for informed technological progress. The thermodynamic properties of materials control their eventual fate in all systems. These considerations are particularly important when the transport of materials through the environment is of concern, as is the case with toxic metals. In addition, the thermodynamic properties of the free radicals involved in transformations of molecules released into the environment may be used to help predict reaction rates and mechanisms.

Approach: Fundamental to our studies of the atmospheric fate of various gases and volatile liquids has been the determination of their atmospheric lifetimes. This has involved the determination of the rate constants for hydroxyl radical reactions with these species by flash photolysis-resonance fluorescence, and the measurement of the ultraviolet cross sections, where appropriate. More recently, we have also estimated solubilities of these gases. Some work has been done on the subsequent reactions of the products of these reactions, and we anticipate that this will become more important in the future.

The kinetics of aqueous-phase transformations of importance to environmental studies have been investigated by use of pulse radiolysis, flash photolysis, gamma radiolysis, and photolysis. These techniques have also been employed to determine thermodynamic parameters for aqueous-phase free radicals.

The primary means we have employed to obtain thermodynamic properties of gas-phase radicals has been the use of photoionization mass spectrometry (in collaboration with researchers at Brookhaven National Laboratory) coupled with theoretical calculations.

Enthalpy increment measurements are made by use of adiabatic calorimetry and by differential scanning calorimetry. Heats of solution will be measured with an adiabatic solution calorimeter, currently being reconstructed.

Results and Future Plans: The investigation of the reactivity of OH radicals with compounds under consideration as replacements for chlorofluorocarbons has continued and a new approach to data analysis has been developed to allow studies of reactions with even lower rate constants than possible previously, yet with high precision. Also, we have continued the measurement of the ultraviolet absorption cross-sections of these compounds. As part of a collaboration with Ford Motor Company, rate constants for the reaction of OH with the proposed fuel additive dimethyl carbonate were measured, and a paper on the atmospheric chemistry of this compound has been submitted. Our studies of the possible atmospheric impact of iodine have culminated in the presentation of a report on the ozone depletion potential of CF_3I.

To provide additional verification of our approach to the determination of atmospheric lifetimes, we participated in a joint study of the reactivity of OH toward CH_2ClBr with scientists from the Russian Academy of Sciences. We employed our flash photolysis-resonance fluorescence approach, whereas they used a discharge flow-electron paramagnetic resonance technique. Both techniques resulted in the same rate constants.

Our investigations in the aqueous phase have focused on transformations taking place in the aerosol phase of the atmosphere. During the year, a major review on this topic appeared in Progress and Problems in Atmospheric Chemistry.

In a new activity on the environmental fate of heavy metals, enthalpy increments of cadmium carbonate were determined. Previous data evaluations resulted in predicted solubilities for cadmium carbonate ranging over three orders of magnitude. The present measurements confirmed a large error in the NBS Tables value for this material. The new results can be used to help predict the fate of cadmium in the environment.

Publications:

Archer, D.G., "***Thermodynamic Properties of Synthetic Otavite, $CdCO_3(cr)$; Enthalpy Increment Measurements from 4.5 K to 350 K,***" J. Chem. Eng. Data 41, 852-858 (1996).

Connel, P.S., Kinnison, D.E., Bergmann, D.J., Patten, K.O., Wuebbles, D.J., Daniel, R. G., Williamson, C.K., Miziolek, A.W., and Huie, R.E., "***Environmental Aspects of Halon Replacements: Consideration for Advanced Agents and the Ozone Depletion Potential of CF_3I,***" Halon Options Technical Working Conference Proceedings, Albuquerque, NM (1996).

Huie R.E., "***Free Radical Chemistry of the Atmospheric Aqueous Phase,***" in Progress and Problems in Atmospheric Chemistry, (John R. Barker, Ed.), World Scientific Publishing Co., Pte. Ltd., 374-419 (1995).

Laszlo, B., Kurylo, M.J., and Huie, R.E., "***Absorption Cross Sections, Kinetics of Formation, and Self-Reaction of the IO Radical Produced via the Laser Photolysis of $N_2O/I_2/N_2$ Mixtures,***" J. Phys. Chem. 99, 11701-11707 (1995).

Orkin, V.L, Huie, R.E., and Kurylo, M.J., "***Atmospheric Lifetimes of HFC-143a and HFC-245fa: Flash Photolysis Resonance Fluorescence Measurements of the OH Reaction Rate Constant,***" J. Phys. Chem. 100, 8907-8912 (1996).

20. Additions to the NIST/EPA/NIH Mass Spectral Database

__S.E. Stein__, C.L. Clifton, W.G. Mallard, A.I. Mikaya (Topchiev Inst. Petrochemical Synthesis, Russia), V. Andreev (Inst. Aircraft Equipment, Russia) R. Mistrik (Univ. Vienna), and B. Yang (Dalian Inst. Chemical Physics, Russia)

Objective: To provide a comprehensive reference database of high-quality mass spectra and software tools to industry and the general scientific community to aid in the identification of a wide variety of chemicals.

Problem: Given the very large number of compounds routinely detected by mass spectrometry, a comprehensive library must contain spectra for a large number of relevant compounds. Maintenance of such a large collection with appropriate quality control is a uniquely challenging task, involving measurement, chemical naming, and a variety of error detection and correction schemes. Moreover, effective use of the database requires specialized tools for dealing with the unique properties of analytical mass spectra.

Approach: Computer methods and human evaluation are the key elements of this program. For database maintenance at NIST, computer methods are used for importing, tracking, and assisting in the evaluation of spectra. For users of the database, tested and documented algorithms are provided for assisting in the identification of unknown compounds. Because mass spectra cannot be predicted to a useful level, every spectrum in the database must be evaluated for its reasonableness by an experienced mass spectrometrist. This involves selection of the best spectra if multiple spectra are available, deletion of questionable spectra, and careful error correction. Other important related activities are the measurement and acquisition of mass spectra, maintenance of collections of chemical structures and retention indices, and close interactions with database distributors and end users.

Results and Future Plans:

Quality: The last data release occurred in 1994 when for the first time all spectra of compounds represented by more than one spectrum were evaluated by mass spectrometrists. Since that time evaluation of the remainder of the database has been the principal focus of this effort. This was completed in late 1995 and since that time recently acquired spectra have been evaluated. The forthcoming release, scheduled for 1997, will be the first which can be said to be fully evaluated, finally bringing its quality up to the standards expected for NIST databases.

Content: Over the past year, a large, high quality collection of approximately 40,000 spectra was obtained and merged with our library. A number of other, smaller collections were also added. The next release is expected to contain at least 50,000 additional spectra, bringing the total to over 120,000. This is by far the largest increase in coverage since the inception of the program and represents a three-fold increase in coverage since the library was transferred to NIST from the EPA some nine years ago.

Software: A new approach for determining the presence and absence of substructures in unknown compounds from their mass spectra has been implemented and released along with the database. Chlorine/bromine determinations as well as molecular weight estimates are also provided. These procedures, in effect, extract relevant substructural information from our library to assist in the analysis of the compounds not represented in our library.

Dissemination: Extensive interactions with distributors and end users have been made to increase awareness of database capabilities. This has been particularly successful this year in that we now expect the majority of the 2,000+ new database installations each year to have full access to all of our mass spectral database products.

Publications:

Stein, S.E., Clifton, C.L., Lias, S.G., Fateev, O.V., Levitsky, A.A., Mikaya, A.I., Levitskaia, T.F., Yang, B., Mistrik, R., Lysak, A.J., Andreev, V.V., and Ausloos, P., ***"NIST/EPA/NIH Mass Spectral Database for Windows, Version 1.5,"*** Standard Reference Database IA (1996).

Stein, S.E., ***"Chemical Substructure Identification by Mass Spectral Library Searching,"*** Am. Soc. Mass Spectrom. 6, 644-655 (1995).

Mistrik, R. and Stein, S.E. ***"Automated Determination of the Consistency of Mass Spectra and Chemical Structure"***, Proc. 44th ASMS Conference on Mass Spectrometry and Allied Topics, May 12-16, 1996, p. 1182.

21. Identification of Chemical Warfare Agents

S.E. Stein, W.G. Mallard, and C.L. Clifton; O. Toropov and A. Rumantsiev (Contractors)

Objective: To develop a highly reliable and fully documented system for the automated detection of chemical weapons and other compounds from data acquired in chromatography/mass spectrometry (GC/MS) analysis.

Problem: The Chemical Weapons Convention (CWC) is a multilateral treaty aimed at eliminating chemical weapons. Verification measures for the treaty call for on-site analysis by GC/MS, with the objective of detecting the presence of prohibited chemicals. However, such a determination would ordinarily require full access to the acquired data, which would present an unacceptable risk of proprietary data loss to many treaty participants. To eliminate this risk, the Department of Defense asked the NIST Mass Spectrometry Data Center to prepare a reliable automated chemical identification program designed to show only information directly related to the aims of the Treaty. An initial version of this program has been developed and successfully employed in equipment validation tests.

Approach: A noise-free spectrum is produced for each chromatographic peak, which is then compared to spectra in a reference library of spectra. Spectral similarity and a variety of other factors are used to produce a "match factor", which is designed to reflect the likelihood that the unknown compound is identical to the reference compound. A variety of established and novel algorithms were implemented for this purpose. A newly acquired collection of tens of thousands of relevant GC/MS data files was used for optimization.

Results and Future Plans: Testing of algorithm performance with thousands of relevant data files indicated that the reliability of chemical identification was comparable to that of an experienced analyst. This was confirmed by actual laboratory trials in four chemical weapons laboratories. Further, evaluation testing on instruments at the headquarters of the Organization for the Prohibition of Chemical Weapons was fully successful, leading to the acceptance of this software for use in CWC verification. Future work will incorporate retention indices, compound class identification algorithms, and testing of instrument operation. A wide range of other applications are being explored for making use of the automation, speed, and reliability of this program.

Publication:

Stein, S.E., Clifton, C.L., Mikaya, A.I., Mallard, W.G., Andreev, V.V., Mistrik, R., and Yang, B. ***"An Automated Chemical Identification System (ACIS) for GC/MS, Version 1.0,"*** NIST (1996).

22. *Database for the Chromatographic Analysis of Alternative Refrigerants*

T.J. Bruno *and K.H. Wertz (Univ. Colorado)*

Objective: To provide reliable and efficient information and methods for identifying and quantifying alternative refrigerants, their synthetic precursors, and potential decomposition products.

Problem: The identification and analysis of alternative refrigerants and their synthetic precursors and decomposition products is a necessary ingredient of thermophysical property measurements; refrigerant synthesis, formulation and manufacture; and the operation of machinery and refrigeration plants. A fast, economical, and reliable method of analysis is necessary for both laboratory applications and for in-line and at-line process analytical determinations. While some spectroscopic approaches offer specific identifications and analyses, chromatography provides the only means to a general scheme. It is also one of the most economical and well-understood methods available.

Approach: We have approached the standardization and automation of the identification and analysis of these materials by measuring standard chromatographic retention parameters as a function of column temperature for the most valuable stationary phases that can be applied to light halocarbons. The standard retention parameters include relative retentions, net retention volumes, and Kovats retention indices. The measurements are performed on a specially modified gas chromatograph that provides highly accurate retention measurements. The column temperature dependencies of these parameters are then modeled with an appropriate mathematical expression to allow prediction at all temperatures. These models are part of an interactive database that allows off-line identification and optimization of more complex analyses. Ultimately, the database will be incorporated into the control software of a process gas chromatograph or used off-line on a PC.

Results and Future Plans: The measurement of all standard retention parameters at four column temperatures (ranging from -20 °C to 16 °C) have been completed for the first stationary phase, a 5% coating of hexafluoropropyl epoxide on graphitized carbon black. Measurements at one temperature have been made on a 5% phenyl polydimethyl siloxane capillary column and on a styrene/divinyl benzene porous layer capillary column. During the next year, all the data on the three stationary phases will be added to the interactive database, and the database will be released. In addition, relative detector response factors have been measured for the thermal conductivity detector.

Publications:

Bruno, T.J. and Wertz, K.H., ***"Retention of Halocarbons on a Hexafluoropropyl Epoxide Modified Graphitized Carbon Black, Part VI: Eleven Fluoroethers and Two Iodofluoroalkanes,"*** J. Chromatography A 736, 175-184 (1996).

Bruno, T.J., Wertz, K.H., and Caciari, M., ***"Kovats Retention Indices of Halocarbons on a Hexafluoropropyl Epoxide Modified Graphitized Carbon Black,"*** Anal. Chem. 68, 1347-1359 (1996).

23. Database for the Chromatographic Analysis of Natural Gas

***T.J. Bruno** and K.H. Wertz (Univ. Colorado)*

Objective: To provide an efficient, fast, and reliable method for identifying in both the laboratory and the field the heavier components of natural gas (the C_6+ fraction, that is, species that have a carbon number in excess of 6).

Problem: The design of many processes involving natural gas, as well as the custody transfer of natural gas, rely on an accurate chemical analysis of the gas composition. However, the most common analysis of natural gas at present considers only the lightest components, and inclusion of the heavier fraction into the analysis (an approach called the "extended natural gas analysis") is a complex problem. In particular, natural gas consists of upwards of 400 organic and inorganic constituents, most of which occur naturally, some of which are introduced intentionally during processing. Moreover, the composition varies with season, with source-well 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.

Approach: Gas chromatography offers an economical and accurate solution to the problem of the extended natural gas analysis. In addition to being one of the most well understood 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 the extended natural gas analysis 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 offline identification of peaks, and also the optimization of more complex analyses.

Results and Future Plans: The measurement of all standard retention parameters at four or more column temperatures has been completed for seven stationary phases. These phases include the well-known methyl silicones and their more useful derivatives, porous polymer and solid adsorbent SCOT (surface coated open tubular) columns, and also some novel stationary phases that include sol/gel phases and clay phases. During the next year, we will add measurements on additional methyl silicone derivatives, and at least one modified adsorbent CLOT (coated layer open tubular) column. All of the modeled data will be entered into the database, which will then be released.

Publications:

Bruno, T.J., ***"Simple and Efficient Methane Marker Devices for Chromatographic Analysis,"*** J. Chromatography A 721, 157-164 (1996).

Bruno, T.J., ***"Sample Storage Devices and Methods,"*** United States Patent, Ser. No. 08/686,426, July 25, 1996.

24. Studies of the Structure and Dynamics of Complex Fluids

***H.J.M. Hanley**, B.D. Butler, C.D. Muzny, G.C. Straty, D.J. Evans (Australian National Univ.), M.Y. Lin and D.G. Peiffer (Exxon Research and Engineering), and S.K. Sinha (Argonne National Laboratory)*

Objectives: To investigate the structure, properties, and dynamics of dense silica gels, clay colloidal dispersions, solutions of worm-like micelles, and micelle/mineral complexes; to develop and improve the metrology used to characterize such systems.

Problems: Despite the technological relevance of gels, colloids, and micellar solutions, a fundamental understanding of their nature is still lacking. For example, silica gel technology is an essential factor in the preparation and fabrication of advanced ceramic materials such as ultra high-purity glasses, yet little is known about the dynamics and structural evolution of the colloidal constituents in the early stages of gel formation. The complex processes involved in the formation and interaction of "worm-like" micelles (which show promise as alternative lubricants) and the interaction of micelles with mineral surfaces (an area of relevance to environmental remediation and clean-up) are also poorly understood. The present lack of understanding of these systems is largely because it is difficult to probe sizes much larger than the atomic scale but still smaller than the mesoscopic scale. Complex interactions that manifest themselves in dense systems are also difficult to model.

Approach: Small-angle neutron scattering, static and dynamic light scattering methods, and computer simulation are the primary tools that we use to better understand and characterize these systems. Our efforts are driven by the recognition that structure, both on molecular and mesoscopic scales, has a predominant influence on the properties of these systems. The small-angle neutron scattering (SANS) spectrometers at the NIST Cold Neutron Research Facility are designed to probe these important length scales and thus play a central role in our research. Static and dynamic light scattering facilities in Boulder are used to augment and extend these SANS results. Unfortunately, it is not always possible to interpret scattering results unambiguously because diffraction processes necessarily result in the loss of some structural information. Here, computer simulations are used to model the experimental system, to aid in the interpretation of the experimental results, and to help suggest which experimental configurations and physical systems are best suited for investigation.

Results and Future Plans: We report here recent results from two of our ongoing studies: 1) the evolution of structural changes during the early stages of gelation, and 2) the interaction of spherical micelles with a clay mineral surface.

Small angle neutron scattering data from dense (30% by mass) gelling 7 nm colloidal silica spheres were obtained using the NIST NG3 SANS spectrometer. We found that the coarsening process that occurs during gelation exhibited temporal self-similarity, and that the time-dependent structure factor obeys a dynamic scaling relation that depends exclusively on a fractal dimension which characterizes the organization of the aggregated colloidal spheres. In an earlier study we demonstrated (using computer simulation methods) how this scaling relation could be applied to the problem of spinodal decomposition in a simple fluid and pointed out the many similarities this simulation has to the problem of gelation in dense systems. Prior to this work it was common practice to extract a characteristic fractal power-law slope from scattering intensities to obtain this fractal dimension, but our results demonstrate that this method will in general fail when the density is high and the aggregates, which ultimately form the gel, interact. The dynamic scaling relation, in contrast, is valid at all densities and should in the future be used instead of the traditional methods to extract structural information from scattering experiments on gels.

Aqueous mixtures of a clay mineral and the common surfactant cetyltrimethylammonium bromide (CTAB) in dilute suspension were investigated by dynamic light scattering (DLS) and small-angle neutron scattering (SANS). Intensity patterns from CTAB/clay suspensions confirmed that CTAB adsorbs on the face of the clay platelets by cationic exchange and that the resulting complex contains excess CTAB in an amount corresponding to at least four multiples of the clay's cation exchange capacity (CEC). The SANS intensity pattern from the suspension of the pure clay is that of non-interacting discs or platelets with diameters of about 30 nm. Contrast matching from the mixture suspensions indicated that the complex retains a disc morphology, even if the CTAB in the aqueous medium is in considerable excess. The DLS data suggest that the suspensions with excess CTAB have effective hydrodynamic diameters in the range 100-200 nm, in apparent contradiction to the smaller size obtained by analysis of the SANS data. From these data we surmised that the suspension consists of a disc-like core with a CTAB interfacial layer which is, in turn, surrounded by a cloud of associated CTAB molecules. The effects of CTAB concentration in solution on the aggregation of the clay and on its subsequent re-dispersion were also investigated.

Plans are to follow up on the two studies listed here and to pursue a program of study which seeks to correlate the interesting rheological properties of worm-like micellar solutions with structural data obtained using neutron and light scattering instruments.

Publications:

Butler, B.D., Hanley, H.J.M., Hansen, D., and Evans, D.J., "***Aggregation of a Quenched Lennard-Jones System Under Shear,***" Phys. Rev. B 53, 2450-2459 (1996).

Butler, B.D., Muzny, C.D., and Hanley, H.J.M., "***Dynamic Structure Factor Scaling in Dense Silica Suspensions,***" J. Phys.: Condensed Matter 8, 9457-9461 (1996).

Lin, M.Y., Hanley, H.J.M., Sinha, S.K., Straty, G.C., Peiffer, D.G., and Kim, M.W., "***Shear-induced Behavior in a Solution of Cylindrical Micelles,***" Phys. Rev. E 53, R4302-R4305 (1996).

Muzny, C.D., Butler, B.D., and Hanley, H.J.M., "***Evolution of the Structure Factor in Gelling Dense Colloidal Silica,***" Mat. Res. Soc. Proc. 407, 87-92 (1996).

Muzny, C.D., Butler, B.D., Hanley, H.J.M., Tsvetkov, F., and Peiffer, D.G., "***Silicate Clay Platelet Dispersion in a Polymer Matrix,***" Mat. Res. Soc. Proc. 435, 85-90 (1996).

25. Advanced Refrigeration Systems for Cryogenic Applications

R. Radebaugh, P. Bradley, E. Marquardt, M. Lewis, T. Kuriyama (Toshiba, Japan), J.H. Xiao (Chinese Acad. of Sciences), C. Walther (Univ. Dortmund, Germany); J. Gary and A. O'Gallagher (Applied and Computational Mathematics Division)

Objectives: To develop new refrigeration techniques and improved refrigerator components for the temperature range of 120 K and below; to investigate and use measurement and modeling techniques for evaluating performance of cryocoolers and their components, such as heat exchangers.

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, the liquefaction of natural gas for clean-burning transportation fuel, and many other existing and potential applications. 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.

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 eliminate most or all moving parts while still maintaining high efficiency. We have focused much of our research on pulse tube refrigerators, which have no cold moving parts. Some applications are now using a recent joint invention of NIST and Los Alamos National Laboratory called a ThermoAcoustically Driven Orifice Pulse Tube Refrigerator, which can produce cryogenic temperatures with no moving parts in the entire system. Mechanically driven pulse tube refrigerators, which have one moving part at room temperature, are more applicable for some other applications where size and efficiency are most important. Experimental and theoretical work at NIST on the behavior of pulse tube cryocoolers and their components yielded efficiencies as high as those obtained with the Stirling cryocooler, which has two moving parts but has been the most efficient of all cryocoolers. Further research is necessary to improve lifetimes and to reduce cost of the pulse tube cryocooler for use in many applications. Our studies encompass measurements and modeling of losses to further improve efficiency while increasing lifetimes and simplifying construction techniques. 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 FY 1996, we have performed measurements on the thermal conductance of stacked stainless steel screen of #400 mesh. Measurements were performed as a function of the helium gas filling pressure ranging from vacuum to 1 MPa. We have found that at the higher pressures (typical of operating cryocoolers), most of the heat is transported by the helium gas across the boundary rather than by the direct metallic contact. The characteristic length through which the heat is transported by the helium gas was found to be in the range of 1 to 4 μm, or about 100 times the mean free path of the helium atoms. The thermal conductance degradation factor for the stacked screen with a porosity of 67% was found to be 0.10. Almost no prior data existed for the thermal conductances of these stacked materials, but they are needed for the optimum design of regenerative heat exchangers in many types of cryocoolers, including the pulse tube refrigerator. Measurements of this thermal conductance as a function of porosity and material have now begun. The results of these measurements will be used to develop a model to predict the thermal conductance.

Under a CRADA with Cryenco, Inc. of Denver, we have collaborated on a redesign of a large pulse tube liquefier for natural gas. This is the world's largest pulse tube refrigerator. The new design used this year should correct the problems with excessive pressure drop in the heat exchangers observed in the original tests last year. A test of one module yielded a no-load temperature of 55 K, compared with 120 K with the defective heat exchangers. A refrigeration power of 300 W at 120 K was observed in this module. A 500 L/day liquefier is now under construction by Cryenco and will be tested in FY 1997 using a thermoacoustic driver powered by burning a portion of the natural gas.

Under a CRADA with Lockheed Martin of Denver, we have collaborated on the design and construction of a miniature two stage pulse tube refrigerator for a space shuttle mission, the date of which is yet to be determined. We are using a commercially available compressor of 0.75 cm^3 swept volume to drive the pulse tube cold head. Power input to the compressor is only 15 W to 20 W. The work under this CRADA involves the world's smallest pulse tube refrigerator. Problems with scaling to small sizes are being studied. To date we have tested a laboratory version using valves for the orifices and obtained a no-load temperature of 127 K with the small compressor and 76 K with a larger compressor. The flight model was constructed using fixed orifices, and tests with it are now just beginning. A few different fixed orifices will be used to find the optimum orifice.

We have used our computer model REGEN 3.1 to compare the performance of regenerative refrigerators with tapered or stepped regenerators with those of straight regenerators. The hydraulic diameter was allowed to vary along the length. The results showed an improvement of only a few percent by using a stepped or tapered regenerator. We had expected a larger improvement. Further studies will be made to better understand the results. We have used our analytical model in deriving the equations that determine the optimum geometry of a tube (we call it the inertance tube) that gives a beneficial phase shift between pressure and mass flow in a pulse tube refrigerator. The effect is only significant in high frequency and/or large pulse tube refrigerators, but in such systems the overall efficiency could be improved considerably. The derivation of these equations will be given in a paper to be prepared next fiscal year. We have designed such inertance tubes for use with the large Cryenco pulse tube liquefier and with an intermediate-size pulse tube refrigerator for use by NASA.

Publications:

Radebaugh, R., "***Advances in Cryocoolers***," Cryogenics (in press).

Ramirez, J.A., Timmerhaus, K.D., and Radebaugh, R., "***Application of the Periodic Temperature Variation Technique to the Measurement of Heat Transfer Coefficients in High NTU Matrices***," Cryocoolers 9 (in press).

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26. Development of the NIST Computational Chemistry Resource

***W.J. Stevens**, K.K. Irikura, and R.D. Johnson*

Objective: To establish databases and other computer resources that will accelerate the use of computational chemistry as a tool for the prediction of molecular properties.

Problem: Computational chemistry software and high-performance computers have evolved to the point where accurate predictions of molecular properties are now possible for molecules containing as many as a few dozen non-hydrogen atoms. In addition, widely-distributed and well-tested computational chemistry software is now accessible to the non-expert. Computation can be the cheapest, fastest, safest, and sometimes the only way to obtain such molecular properties. However, the usefulness of computational chemistry is limited by uncertainties about the accuracy and reliability of the diverse computational methods that may be applied to a particular problem. Also, non-experts need guidance in the use of computational chemistry software and in the development of strategies for attacking problems in molecular structure and properties.

Approach: The Physical and Chemical Properties Division has initiated a program to establish the NIST Computational Chemistry Resource (CCR) to aid industrial scientists who want to compute molecular properties. This resource will enable the assessment of accuracy and reliability of computational chemistry methods and will provide non-expert scientists with the tools they need to develop strategies for the application of computational chemistry. The CCR will be integrated eventually with the NIST Chemistry WebBook also being developed by the Division (see Report 1).

Results and Future Plans: The initial design for the CCR consists of three parts: 1) A database of critical comparisons of computational results with the best available experimental data will focus, initially, on heats of formation. This database will allow the accuracy and reliability of computational methods to be assessed for specific classes of chemical compounds. For the initial stages of this work, a collaboration has been established with the DoE Pacific Northwest National Laboratory. 2) A national repository will be established for archiving the results of quantum chemistry calculations. The archive will be set up as a database with a sophisticated graphical user interface. This will allow, for the first time, previous computational results to be searched and compared by scientists who are contemplating calculations on new molecular species. 3) A sophisticated graphical user interface will be developed that, eventually, will provide interactive guidance to the non-expert on the choice of methods, predicted accuracy and reliability, and resource requirements. Collaborative arrangements are being explored.

VI. Analytical Chemistry Division (839)

Willie E. May, Chief

A. Division Overview

The Analytical Chemistry Division serves as the Nation's Reference Laboratory for chemical metrology 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 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, precision, sensitivity, and selectivity. The resulting reference methodology is used in a highly- leveraged manner and serves as the foundation for the certification of chemical composition in more than 850 Standard Reference Materials important to U.S. industry, government agencies, and educational institutions. Measurement methods derived from Division research activities are also used for establishing and maintaining chemical measurement traceability links for commercial reference materials producers and measurement comparability links with chemical metrology laboratories world-wide. Congressionally appropriated funds constituted 60% of the Division's FY 1996 operating budget. The remainder came from other federal and state government agencies and American industry for research and/or measurement services provided on a cost reimbursable basis.

Chemical measurement science-related research activities within the Division are carried out in the following areas:

- analytical sensing technologies
- classical analytical methods
- gas metrology
- laboratory automation technology
- nuclear analytical methods
- organic analytical methods
- spectrochemical measurement methods

Our vision is to leverage the skills and knowledge gained from these activities to establish and maintain the infrastructure to provide U.S. industry and the Nation with:

- tools for achieving international comparability of chemical measurements through
    - *high priority Standard Reference Materials (SRMs)*
    - *NIST-traceable reference materials from commercial sources (NTRMs)*
    - *interlaboratory proficiency testing programs in critical areas*
    - *intercomparisons of primary methods and standards with other national metrology laboratories*
- critical analytical data and specialty analyses.

In terms of organization, the Division consists of five organizational subunits/Groups:

- Spectrochemical Methods
- Organic Analytical Methods
- Gas Metrology and Classical Methods
- Chemical Sensing and Automation Technology
- Nuclear Methods

The brief group overviews provided below and Selected Technical Reports that follow illustrate the synergistic relationship among the Division's research activities in chemical measurement science, its standards and quality assurance activities, and new programs in the analytical reference data area.

Spectrochemical Methods

Research activities in this area are focused on the development, critical evaluation, and application of methods for the identification and measurement of inorganic chemical species using x-ray, optical, and mass spectrometries. For example, new procedures for the evaluation of the magnitude and reliability of blank measurements in isotope dilution thermal ionization mass spectrometry (ID-TIMS) have allowed high accuracy measurements of sulfur in solids to be carried below the 1 μg/g level for the first time. The impetus for this research comes from the aerospace industry through the NIST Aircraft Engine Casting Consortium. The aerospace industry has had problems in achieving a consistent bond between the thermal barrier coating and the high temperature alloy of the jet engine turbine blade. It is currently believed that the difference between 0.2 µg/g and 1 µg/g of sulfur in the superalloy represents the difference between acceptable and unacceptable materials. Using the new blank evaluation procedure, sub-µg/g sulfur determinations in turbine blade alloys have now been demonstrated by ID-TIMS. Measurements have also been completed resulting in the recertification of SRM 131e, **High Silicon Low Alloy Steel**. This material is presently certified for sulfur at a concentration of 4 µg/g ± 2 µg/g. Despite the high relative uncertainty, it is heavily relied on by the metals industry for calibration of low sulfur determinations. The new measurements now place the value at 4.34 µg/g with an expanded uncertainty of 0.10 µg/g.

IDMS research using inductively coupled plasma mass spectrometry (ICPMS) has concentrated on approaches to improving the efficiency and ease of analysis, along with instrumental improvements in the performance of the technique. A commercially-available automated on-line chemical separation system has been installed and evaluated. This system makes it easier for us to take advantage of one of the primary features of quantitation by isotope dilution — the ability to eliminate interferences through chemical separations without concern for quantitative analyte recovery. A direct-insertion nebulizer has also been tested for use with ICPMS, leading to improved signal stability, reduced memory effects, and decreased sample usage.

UV/Visible Fourier Transform Spectroscopy continues to be developed as a technique for high-accuracy wavelength measurements. Wavelength measurement uncertainties using the two-color laser wavelength calibration technique developed last year at NIST have now been evaluated in detail, and work will begin in FY 1997 to measure new ^{198}Hg wavelength standards. The emphasis of research in the area of glow discharge optical emission spectrometry has shifted to the determination of non-metals in the vacuum UV, an area of weakness in present analytical capabilities. A limit of detection for phosphorus of 2 µg/g in metal alloys has been demonstrated using a purged-gas spectrometer. X-ray fluorescence (XRF) has been used to characterize TiN thin films on silicon prototype reference materials. Information on the thickness, uniformity, purity, density, and stoichiometry of the thin film was obtained. The semiconductor industry, through SEMATECH, has requested the development of thin film SRMs.

Definitive IDMS methods have been applied this year to the determination of Ca, Mg, Li, and K for the certification of SRM 956a, **Electrolytes in Frozen Human Serum**. This SRM is intended for use as a calibration standard in clinical laboratories, thus establishing NIST traceability for important health-care diagnostic markers. Calibration SRMs benefit greatly from the very high precision and accuracy afforded by IDMS. The full range of optical, x-ray, and mass spectrometric techniques available in the group were applied in the past year to the certification of inorganic constituents in reference material matrices such as human serum, household dust, paint powder, soils, bone phantoms, oils, industrial sludge, metals on filters, and spectrometric solutions. The Environmental Protection Agency (EPA) sponsored the certification of powdered paints, dusts, and two soil reference materials to support the reduction of lead contamination in the U.S. We are also working with EPA as part of the National Human Exposure Assessment Survey to provide analytical reference laboratory support for trace metal analysis.

During the past year, we also collaborated with the Desert Research Institute to determine sources of pollution which may be responsible for the degradation of visibility in the Mt. Zirkel Wilderness Area of Colorado. Our work involving precise sulfur isotopic measurements of airborne particles is described later in more detail in Technical Report #3.

Organic Analytical Methods

Activities within this group are directed toward the development, critical evaluation, and application of methods for the identification and measurement of organic and organometallic species. Currently, laboratory research activities are focused on organic mass spectrometry and separation techniques including 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 electrospray ionization as a tool for the quantitative determination of proteins in biological matrices. A method for the mass spectrometric determination of albumin (M_r [relative molecular mass] 65K) in serum has been developed and demonstrated good agreement with a standard protein assay. We are currently working toward establishing reference methods for other important biomarkers such as glycohemoglobin (an important marker for diabetes) α-fetoprotein (a marker for liver disease), serum thyroxine (an important marker for thyroid function), and cortisol. Development of accurate methods for the latter two analytes is an important component of our ongoing collaboration with the College of American Pathologists (CAP) to establish NIST traceability for important health markers.

Recent research activities in separation science have focused on investigations of the physical and chemical processes that influence the separation and detection of organic compounds, and the application of these techniques in the development of reference methods for the determination of these compounds in natural matrices. We are investigating the molecular interactions that influence chromatographic retention with particular emphasis on the design of stationary phases for specific applications in LC, GC, and SFC. In LC, our recent efforts have focused on the synthesis and characterization of C_{30} and C_{34} alkyl chain stationary phases specifically tailored for the difficult separation of β-carotene isomers and other carotenoids of interest in health and food/nutritional studies. Current research in chiral separations is focused on developing SFC separation strategies for optical isomers of pharmaceutical and agricultural interest. We have demonstrated that SFC provides significant advantages over the LC-based methodologies currently used. Of particular interest is the use of coupled chiral and achiral columns in SFC to achieve separations that would not be possible in LC. Our research in organometallic "speciation" has resulted in the first certified values for methylmercury in three mussel tissue SRMs using a method based on gas chromatography with atomic emission detection. Efforts in organometal speciation are continuing with emphasis on arsenic speciation.

Several new SRMs are being developed to support measurement activities in the environmental, health-care, food, and nutritional labeling communities. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated pesticides, and methylmercury have been certified in a **Contaminated Marine Sediment** (SRM 1944) and three **Mussel Tissue** materials (SRMs 1974a, 2974, and 2976) to support marine environmental monitoring efforts. Two new SRMs related to diesel emissions, **PAHs in Diesel Particulate Matter** and **Extract** [SRMs 2975 and 1975], were developed to provide an accuracy benchmark for both the chemical measurement and Ames bioassay communities. Measurements were completed for certification of SRM 1951a, **Lipids in Frozen Human Serum**, which will be used by the clinical laboratory community to establish an NIST traceability link for important heart disease risk markers such as total, HDL-, and LDL-cholesterol, and triglycerides. Blood glucose is an important diagnostic marker for diabetes and has been shown to undergo slow degradation (~1%/yr) in lyophilized serum; SRM 965, **Glucose in Frozen Human Serum**, was developed to address this problem. The certification of an **Infant Formula** [SRM 1846], a **Baby Food Composite** [SRM 2383], and a **Human Diet Composite** [SRM 1544] were completed with values assigned for a number of vitamins, minerals, fat (as fatty acids), and proximates (carbohydrates, protein, fat, etc.). These SRMs will be used by the food testing/nutritional laboratory community to facilitate compliance with new nutritional labeling laws and to provide accurate labeling information to assist consumers in making sound nutritional choices. Dr. Stephen A. Wise received an FY 1996 Measurement Services Award for establishing the joint public-private sector research and measurement programs that have led to the rapid development of these SRMs.

Measurement quality assurance services are provided to support several major other Federal Agency programs. We serve as an analytical reference laboratory and Quality Assurance Center for approximately 60 laboratories world-wide involved in studies (National Cancer Institute) to determine the cancer preventive benefits of dietary intakes of selected micronutrients to high risk human populations and for over 40 laboratories involved with the measurement of organic contaminants in marine sediments and tissues as part of various marine monitoring programs (National Oceanic and Atmospheric Administration and the Environmental Protection Agency). We are interacting with eight laboratories involved in the measurement of heavy metals, pesticides, and PAHs in soil, water, food, and indoor/outdoor air samples collected and analyzed as part of the EPA National Human Exposure Assessment Study. We are also working with the National Institute of Justice on the development of methods and standards for the analysis of drugs-of-abuse in hair, and with Department of Defense on the development and critical evaluation of the chemical measurement protocols to be used during industrial onsite inspections for monitoring compliance with the pending Chemical Warfare Agent Treaty.

Gas Metrology and Classical Methods

Research activities within this group are focused on gas metrology, classical wet chemical methods (gravimetry, titrimetry), coulometry, ion chromatography, optical spectroscopy, and maintenance of the theoretical infrastructure for pH and conductivity measurements.

We are assisting the U.S. automobile industry in developing and evaluating new analytical measurement tools and standards needed to address compliance issues associated with the Clean Air Act of 1990. We are developing an infrared-based sensing device and working with the NIST Physics Laboratory to evaluate a new microwave device for detection of oxygenated hydrocarbons in automobile exhaust. The use of oxygenated and reformulated fuels has been required by a number of metropolitan areas in order to comply with Federal air quality standards. Standards that are currently under development include a suite of synthetic gasoline materials being certified for benzene, total aromatics, total oxygen, total olefin, and sulfur content. These SRMs will provide NIST traceability for the new reformulated gasolines. Another related standards activity involves the development of catalytic conversion technology for the on-demand generation of standard formaldehyde-in-air mixtures from methanol-in-air standards. A prototype device is currently being field tested in an automobile industry facility.

In addition to the standards activities mentioned above, 25 cylinder gas, 44 spectrometric solution, 4 conductivity, and 4 pH SRMs were certified during the past year and 88 cylinder gas SRMs were submitted by customers for recertification. While these standards are critically needed, their continuous production consumes considerable fiscal and human resources and limits our ability to address new standards needs. A **N**IST **T**raceable **R**eference **M**aterials Program has been conceptualized to address this issue. An NTRM is a reference material, with a well-defined traceability linkage to existing NIST standards, produced and sold by a commercial supplier. This linkage is established via criteria and protocols defined by NIST to meet the needs of the metrological community to be served. The gas NTRM program was implemented in 1992 in partnership with EPA and specialty gas companies as a means for providing end-users with the wide variety of certified gas standards needed to implement provisions of the 1990 Clean Air Act. Gas NTRMs are produced and distributed by specialty gas companies with NIST oversight of the production and involvement in the analysis, and can be developed for any compound whose concentration is bounded by a NIST primary standard suite. Certified concentration values are assigned by NIST according to a published protocol. Since 1993, eight commercial vendors have produced over 2900 NTRMs which were used to generate more than 150,000 commercial 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.” Plans are being put in place to extend the NTRM model to other sectors of the commercial reference materials community with initial emphasis on spectrometric solutions.

Structured intercomparison programs with other National Metrology Laboratories continue to be our basis for formally establishing equivalence of primary methods and standards important for world trade and commerce. During FY 1996, the "Declaration of Equivalence" for primary gas standards between NIST and the Netherlands Measurements Institute was expanded to include seven primary mixture suites. A new Memorandum of Cooperation was signed with the Danish Institute of Fundamental Metrology and the Hungarian National Office of Measures for intercomparison of conductivity primary standards. Talks were initiated concerning intercomparisons between NIST and the European Union in the area of pH. More information concerning these activities can be found in the Technical Highlights Section that follows.

We have been working with pediatric researchers and scientists from the National Institute of Child Health who are evaluating the therapeutic value of inhaled nitric oxide in newborn patients. We have also initiated a program to support EPA's protocol TO-16, quantitative FT-IR open path monitoring of industrial air pollution. Both these programs are further described in the Technical Highlights Section.

Chemical Sensing and Automation Technology

Activities in this Group (CS&AT) are directed toward the development and application of new technologies, techniques, and standards for chemical sensing, sample preparation, and laboratory automation for chemical analysis. These activities are precursors to the development of new techniques and technologies that benefit U.S. industry, scientific organizations, and other government agencies and that provide foundations for standards development activities which benefit producers and consumers world-wide.

A candidate material for a glass near-infrared (NIR) wavelength standard (700 to 1700 nm) was developed based on previous studies in the Group. This year a commercial "brick" of this rare-earth-doped glass was obtained and approximately forty 25-mm round disks filters were cut from this material and polished. These filters were evaluated for wavelength reproducibility, stability over a limited temperature range, and for acceptable transmission characteristics using our Fourier Transform NIR spectrophotometer. Currently, in collaboration with the Optical Technology Division, we are determining the positions of selected peaks and possible polarization effects using a dispersive spectrophotometer. For this SRM, the "center-of-gravity" method will be used for the actual certifications (as specified by ASTM and previously used for SRM 1921 in the mid-IR range) because of its performance with asymmetrical peaks. Information values using other algorithms currently popular with instrument vendors will also be provided in the certificate. A round robin activity is being scheduled to afford potential customers an opportunity to evaluate and comment on the candidate filter material before we go into full production of the SRM.

We recently held a workshop with the clinical chemistry and instrument manufacturing communities to determine and prioritize needs for low-level and single-photon-counting light intensity standards. It was determined that two types of standards need to be developed initially: low-level artifact standards that permit traceability to the SI base units and standard chemical reactions that produce a known number of photons per mole. The latter standard could be used to calibrate instruments, such as flow-through devices, where an artifact standard would not be useful. Parametric evaluations of candidate technologies for the artifact standards are in progress.

After five years of effort to develop standards for laboratory automation, the Consortium on Automated Analytical Laboratory Systems (CAALS) has formally gone out of operation. In its wake, CAALS leaves an architecture for automated laboratories, a specification of expected and acceptable behaviors for instrument components of automated systems, a draft standard for instrument-to-controller communications (CAALS-I), a high-level communication protocol (HLCP), a common command set, and a mechanism for representing instrument features and idiosyncrasies know as an instrument capability dataset. Currently, these CAALS concepts are being utilized in other government agency projects, university research programs, and commercial ventures. CAALS work is the basis for a draft standard currently being balloted by ASTM and will likely form the foundation for new standards being undertaken in the clinical chemistry automation community under the auspices of the National Committee for Clinical Laboratory Standards.

For over twenty-five years, we have provided optical filters to calibrate the transmittance and wavelength scales of visible and ultra-violet spectrophotometers. The records of each filter's certification and (sometimes numerous) recertifications have been carefully preserved. These data contain a wealth of information about filter stability and long-term performance, but unfortunately, it is very difficult to extract information from their present paper format. We have created a database that will contain all the information about an optical filter SRM. New data created during filter certifications and recertifications are automatically entered into the database. A mechanism being developed to scan in the existing paper records using an optical character recognition program will eventually allow us to have the data from all 25 years available electronically. This will allow us to understand better the aging effects on the filters' transmittances and to determine with better statistical certainty the appropriate intervals between recertifications, not to mention the improvements in efficiency by automating the data verification, report generation, label printing, and accounting functions of the program. In the future, using the database will give us better understanding of our filters and will improve operation of our optical filters production and recertification efforts.

Additional research activities concerning the rapid determination of gasoline additives by combined FT-IR/FT-Raman spectroscopy (#4), chemometric methodology for evaluating DNA data used for human identification (#5), and a new tool for the qualitative and quantitative chemical characterization of liposome preparations (#15) are described in the Selected Technical Reports that follow.

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 also that they often are nondestructive and thus do not require sample dissolution.

We have conducted research in NAA at NIST for many years, and new developments continue to provide improvements in detection sensitivities, elemental specificity, precision, and overall accuracy. This year, a new pneumatic tube has been installed into the NIST nuclear reactor. This fast pneumatic tube propels the sample out of the irradiation facility and into the counting location in 0.5 second, and enables the measurement of irradiation, decay, and transfer times to within 0.01 second. This new system will provide NIST with the capability for accurately determining elements with very short half-life activation products such as fluorine (using fluorine-20; half life=11.0 s) which is difficult to determine at low levels, selenium (using selenium-77m; half life= 17.4 s) which can be determined much more rapidly than the long lived selenium-75, and possibly, lead at elevated levels (using lead-207m; half life = 0.8 s). This new facility complements the existing state-of-the-art capability for high accuracy instrumental and radiochemical NAA. During the past year, Donald A. Becker was elected as a Fellow of the American Nuclear Society "For the development of high accuracy methods and quality assurance techniques in neutron activation analysis, and the application of these methods and techniques to the certification of trace element reference materials".

Pioneering research is conducted within this Group on the use of neutron beams as analytical probes using 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 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 the cold neutrons from the NIST reactor.

After an extended reactor shutdown, the cold–neutron PGAA and NDP spectrometers have been rebuilt to take advantage of the newly installed cold neutron source at the NIST reactor. This new moderator is a spherical shell of liquid hydrogen 32 cm in diameter and 2 cm thick. Its enhanced efficiency and lower operating temperatures (in comparison with the old D_2O ice source that had been in use since 1987) have resulted in both higher neutron flux and longer wavelengths in the beam. The new analytical instrument designs, in combination with the new cold source, have resulted in detection limit improvements of about a factor of four for both instruments.

Efforts continue within the group to develop the techniques and methodology of focusing cold neutron beams for analytical applications. A recent focusing lens using capillary fiber optics takes a beam of cross section 50 mm x 45 mm from a guide viewing the reactor cold source and focuses it to a small spot of 0.5 mm diameter. This reduction in size gives an increase by a factor of 80 in the neutron density relative to the incident beam. This optic has been used in conjunction with PGAA, and has improved the sensitivity by a factor of 20 for certain elements. Further efforts have been made with a bender-focuser lens to produce the focused spot in a region outside the shadow of the incident beam, which will substantially reduce the background as well. Moreover, initial test measurements have been made on monolithic tapered lenses which are preferable for certain instruments such as NDP. Additional research is being performed on the use of metal capillaries which might increase the transmission efficiency of these lenses.

Future Directions

All Division research and service projects are reviewed on an annual basis for quality and match-to-mission as well as to customer needs. The general focus of the research and measurement service activities within the Division will not change very much in the coming year except in our Chemical Sensing and Automation Program, where our attention to measurement and standards needs in the area of process analytical chemistry will increase. This increased emphasis on real-time measurement and standards issues is reflected in the following list of new projects that will be initiated over the next two years.

- Sample Modulation in Atomic Spectrometry
- On-Line Coulometry for Process Applications
- Packed Column Capillary Electrokinetic Chromatography
- Compositional Mapping by Neutron Depth Profiling Using a Neutron Probe from a Monolithic Lens
- Mass Spectrometry as a Tool for Characterization and Standardization of Clinical Immunoassays
- Quantitative Gas-Phase Database for FTIR Open-Path Sensing Applications
- Combined NIR/Raman Instrumentation for Process Analytical Measurements
- Low-Level Light Standards for Chemiluminescence and Other Photon Counting Detectors
- As and Hg Speciation in Marine SRMs
- Methods and Standards for Oxygenated Hydrocarbons in Automobile Exhaust

The Division's budget for Standard Reference Materials development 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. During the past two years, we have been able to initiate efforts for development of a number of high priority new SRMs including the following examples:

- Reformulated Gasolines *(Oxygenates, organic and total sulfur, and aromatics to be certified)*
- Hydrogen in Titanium *(Brittleness of aircraft turbine blades related to H_2 concentration)*
- Zeolite Catalysts *(Na, Si, Al, Fe, and trace metals to be certified)*
- Aircraft Alloys *(P and S to be certified; these elements related to materials failure)*
- NIR Wavelength *(for precise calibration of spectral region of increasing industrial interest and use)*
- Low-Level Nitric Oxide *(for NO/NO_2 sensing devices used to monitor emissions from "clean cars")*
- Canned Meat for Nutrition Labeling *(Cholesterol, fatty acids, and minerals to be certified)*
- Organics in Fish Tissue *(Toxic contaminants such as PCBs, pesticides, and methylmercury to be certified*

- Heavy Metals in Blood (*Toxic metals such as Cd, Pb, total mercury, and methylmercury to be certified*)

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 Reports and Outputs and Interactions sections 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 support for environmental quality and human health assessments.

B. Selected Technical Reports

1. *Measurement Methods and Standards in Support of New Inhaled Nitric Oxide Therapy*

W.J. Thorn III and P.M. Chu

Objective: To assist pediatric researchers and physicians at the National Institute of Child Health and Development (NICHD) and the Food and Drug Administration (FDA) in developing guidelines, accurate gas standards, and protocols for evaluating medical devices needed to implement a new inhaled nitric oxide (INO) therapeutic regimen.

Problem: Clinical trials on INO supervised by the NICHD have been concluded and it is expected that the FDA will soon approve the use of INO therapy for newborn patients. Preliminary results from these trials indicate that when inhaled with oxygen via a ventilator, dilute concentrations (<100 µmol/mol) of NO have shown dramatic results as a pulmonary vasodilator in some newborn patients.

NO_2 can cause pulmonary edema and other problems in the patient's respiratory system. NO_2 is always present in the INO ventilator gas. It is a major contaminant in the nitric oxide source gas and NO_2 is spontaneously formed when NO mixes with oxygen in the ventilator circuit. The amount of NO_2 formed is a function of the system dwell time (the time it takes the blended NO/oxygen mixture to reach the patient). Because of this potential for adverse effects, levels of NO and NO_2 must be continuously monitored in a typical matrix of >0.8 mole fraction of oxygen during the therapy. The FDA Anesthesiology and Respiratory Devices Therapy Panel would like to limit the maximum allowable concentration of NO_2 in the patient's ventilator circuit to 1.0 µmol/mol. Measurement of NO_2 at this level in an INO ventilator circuit by normal NO/NO_2 devices such as chemiluminescence or electrochemical sensors is difficult in this oxygen-rich matrix. Gas standards of known concentration, emulating INO ventilator circuit mixtures, are needed to properly evaluate device measurement behavior.

Approach: NIST primary gas standards served as the basis for the preparation of the series of nitric oxide mixtures used in the study. NIST/NICHD collaborators used tuneable diode laser absorbance spectrometry (TDLAS), capable of measuring >0.02 µmol/mol NO_2 in the INO matrix, to study the kinetics of the formation of NO_2 as a function of system dwell time. NICHD researchers also used NIST TDLAS technology to benchmark the performance of new devices designed to measure NO and NO_2 in high-oxygen environments.

Results and Future Work: For the past two years, we have been working with NICHD to understand the kinetics of NO_2 formation under conditions expected to be used in INO therapy. The rate of NO_2 formation under these conditions was determined to be 0.137 µmol/mol for each second of dwell time at the clinically significant conditions of 80 µmol/mol NO in a 0.9 mole fraction oxygen matrix. It was concluded that no significant levels of NO_2 should reach the patient during treatment at anticipated dwell times of <0.5 second.

A prototype facility has been established at NIST to allow sensor and instrument manufacturers to evaluate the performance of their devices for measuring NO and NO_2 under simulated therapeutic conditions. During a recent workshop held at NIST for pediatric researchers, technical experts used this facility to test new chemiluminescence monitors and solid-state sensor devices designed to perform in high-oxygen environments. The performance of these devices was far superior to those evaluated at NIST a year ago and additional workshops are planned to assist the FDA in further evaluations. We have also collaborated with a commercial specialty-gas company in the development of NIST-traceable gas mixtures for calibrating the NO/NO_2 sensors to be used in this new therapeutic regimen.

2. *Spectral Reference Database to Support Open-Path FT-IR Measurements*

P.M. Chu, G.C. Rhoderick, D.L. VanVlack, F.R. Guenther, and W.J. Lafferty (Optical Technology Division, PL)

Objective: To develop a reference spectral database to support open-path Fourier transform (FT) infrared measurements of hazardous air pollutant (HAP) molecules.

Problem: FT infrared-based technologies are being developed for real-time monitoring of airborne chemical contaminants within plant facilities and along boundaries. Recognizing the potential of FT infrared methods, the Environmental Protection Agency (EPA) is currently preparing a formal protocol (Method TO-16) for FT infrared open-path remote sensing. The success of this program is highly dependent on the availability of high-quality reference spectral data. This collaboration between NIST and EPA will provide industry with a tool for regulatory compliance that is both cost effective and less invasive than current practices

Approach: Working standard mixtures are prepared at NIST using starting materials of assessed purity and procedures that minimize contamination. These mixtures are benchmarked by gas chromatography against NIST primary gas standards.

The mixtures of the target contaminants in air, at various known concentrations, are each continuously flowed through the sample cell and data averaging is initiated after a steady-state spectrum is obtained. This minimizes biases caused by sample adsorption to the cell walls. A research grade FT infrared spectrometer is used to obtain the spectra at a nominal resolution of 0.120 cm^{-1}. Software is being developed to degrade the spectral resolution to match that of field instruments. The National Physical Laboratory (UK) as well as a number of expert laboratories in the United States will also provide spectral data using the NIST-prepared gas mixtures.

Results and Future Plans: Our initial work focused on examining a number of the instrumental parameters such as apodization functions, resolution, wavelength calibration, detector properties, and instrument stability. In the FT infrared data acquisition protocol developed for each contaminant, nine spectra are acquired at 0.120 cm^{-1} resolution using three different concentrations and three different path lengths. In each case, the interferogram is transformed using a three-term Blackman-Harris apodization function, Mertz phase correction, a zero-filling factor of two, and a non-linear correction routine provided in the software package supplied with the instrument. Using the known concentrations and path lengths, point-by-point absorption coefficients are calculated and tabulated on a grid of 20 points/cm^{-1}. In collaboration with EPA and industry, we have identified the 18 highest priority HAPs from the 189 compounds listed in the EPA Clean Air Act Amendment of 1990 for our initial focus.

Publication:

Chu, P.M., Rhoderick, G.C., Lafferty, W.J., Guenther, F.R., and Wetzel, S.J., **"A Quantitative Infrared Database of Hazardous Air Pollutants,"** Proceedings of the Air & Waste Management Association, 89th Annual Meeting and Exhibition, Nashville, TN, June 23-28 (1996).

3. *Isotopic Analysis of Source and Receptor Samples for the Mt. Zirkel (Colorado) Wilderness Area*

W.R. Kelly, R.D. Vocke, and T.L. Quinn

Objective: To measure the natural variation in the $^{34}S/^{32}S$ isotopic ratios in atmospheric source and receptor samples collected from December 1994 through November 1995 near the Mount Zirkel Wilderness Area (MZWA) in northwestern Colorado. This was a collaborative project with the Desert Research Institute of the University of Nevada, Reno to determine sources responsible for visibility degradation in the MZWA.

Problem: The reduced visibility in the MZWA has been linked to suspended sulfur-bearing particles emitted from stationary sources in the surrounding areas. By law, any loss of visibility due to anthropogenic activity must be remediated. Current information is inadequate to allow "reasonable attribution" of the observed visibility impairment to specific sources. However, if sulfur isotopic ratios were sufficiently different for different sources, then measured ratios along with other chemical data for receptor samples could be used as differentiating components in source apportionment models.

Approach: The $^{34}S/^{32}S$ isotopic ratios were measured in approximately 60 source and receptor samples by thermal ionization mass spectrometry (TIMS). The sulfur-emitting sources sampled in this study include two separate coal-fired power plants, vehicle exhaust, geothermal hot springs, forest fires, residential wood burning, and residential coal burning. Many of the samples contained less than 100 µg of sulfur. At these levels, only the TIMS technique developed at NIST is capable of providing the sensitivity and precision required to resolve this problem.

Samples were received as sulfate in aqueous solution. The sample sulfate was reduced to hydrogen sulfide, trapped in ammonia, and then precipitated as As_2S_3. The $^{34}S/^{32}S$ ratio was measured as $As^{34}S^+/As^{32}S^+$ molecular ions in a 30 cm radius of curvature, 90° sector thermal ionization mass spectrometer of NIST design and construction. Replicate measurements indicate a reproducibility characterized by a relative standard deviation of 0.2%.

Results and Future Plans: Isotopic measurements are usually expressed as deviations in parts-per-thousand from an internationally accepted standard. The standard for sulfur is S-1, a silver sulfide, which defines a delta scale in terms of per mill (‰) deviations from Vienna Canyon Diable Troilite (V-CDT), or $\delta^{34}S_{V\text{-}CDT}$.

Figure 1 summarizes the variation of delta values for the various sources and receptor/ambient air samples. The width of the dots corresponds to the estimated uncertainty (2 ‰) of the measurement. The ambient air samples vary between +2 ‰ and +7 ‰ and show a significant overlap with the power plant signatures, implicating them as a possible source of sulfate in the MZWA. The only other isotopically heavy sulfur sulfates originate from residential wood and residential coal burning which contribute much less to the total emissions. The isotopically lighter portion of the ambient air signature points to a source other than the power plants and probably involves the contribution of a mix of sulfates from geothermal and vehicular exhaust sources.

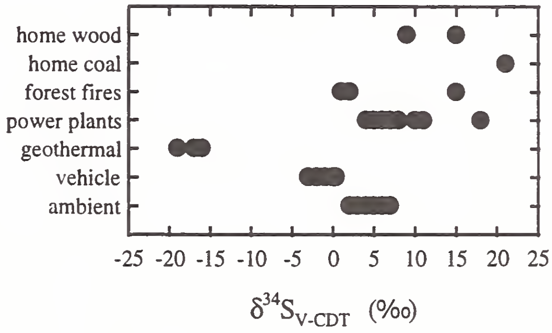


Figure 1. Variation of $^{34}S/^{32}S$ isotope ratios from various sources in the Mount Zirkel Wilderness Area, expressed as $\delta^{34}S_{V\text{-}CDT}$

Future plans are to use this TIMS technology to measure sulfur isotopic ratios in polar ice cores. A comparison of isotopic signatures in pre-industrial (pre-1760) and recent core samples may allow us to distinguish more clearly between anthropogenic and natural sources of atmospheric sulfur, and possibly assess the impact of anthropogenic sulfate emissions on the global distribution of sulfur. Sulfate concentrations in ice cores typically range from 90 to 200 µg/kg.

4. FT-Near IR and FT-Raman Technology for On-Line, Real-Time Analysis of Oxygenates in Fuels

S.J. Choquette, S.N. Chesler, and D.L. Duewer

Objective: To develop a non-invasive method for determining mixed oxygenates in ampoules of gasoline that can be used to assure quality in NIST Standard Reference Materials (SRMs) and NIST Traceable Reference Materials. This technology is also being evaluated for its potential as an on-line method for monitoring refinery blending operations.

Problem: Oxygenated fuels, designed to lower CO emissions, are gasolines blended with alcohol or ether additives. NIST provides eight oxygenated gasoline SRMs each containing one of the four oxygenates: ethanol (ETOH), methyl *tert*-butyl ether

(MTBE), *tert*-amyl methyl ether (TAME) and ethyl *tert*-butyl ether (ETBE). These SRMs are provided in 20-mL sealed glass ampoules containing one of the four oxygenates at the 2.0 or 2.7 % oxygen mass fraction level. The current method employed to certify these materials is capillary gas chromatography (GC) with flame ionization detection. Although this separation-based technique provides excellent precision and accuracy, it requires opening the ampoules. As a result, these SRMs must be batch-certified with the expanded uncertainty of their assigned values incorporating an estimate of batch heterogeneity. While the current expanded uncertainty for all these SRMs is an acceptable 0.04 to 0.06 % mass fraction oxygen, the batch heterogeneity component is attributable to only a few "outlier" ampoules. These statistical outliers are thought to be due to errors in the flame-sealing procedure and/or sample handling. The expanded uncertainties for all such SRMs could be lowered if each individual SRM ampoule could be characterized and obvious "outliers" discarded from the batch. This requires a non-destructive, rapid, and precise analytical method.

Approach: We previously reported on the use of Fourier Transform-Near Infrared (FT-NIR) and Fourier Transform-Raman spectroscopic methods to determine the oxygenate content of gasoline SRMs without opening the ampoules. This work is continuing with the development of a hybrid approach that utilizes both methods for on-line, real-time analysis.

Results and Future Plans: Both Raman and NIR methods were shown to be capable of quantifying the oxygenate concentration to within the expanded uncertainty of the destructive GC measurement. In addition, measurement of the oxygenate concentration in an individual ampoule was accomplished in less than 1 minute. Although the results obtained with the NIR method gave better estimates (lower standard deviations), the Raman method was more capable of identifying the oxygenate in unknown data sets. For the analysis of complex mixtures, a dual mode instrument would have the advantage of the selectivity of the Raman and the precision of the NIR while avoiding the time consuming manual transfer of ampoules from one instrument to the next. Due to the real-time requirements of the experiment, a dual NIR/Raman instrument was conceived and is being evaluated. To implement this approach we added a fiber-coupled tungsten source to the FT-Raman instrument. Because the FT-Raman instrument uses a 1.06 µm source, the Raman-scattered light is emitted in the 10,000 cm^{-1} to 5,000 cm^{-1} (absolute) region. This corresponds to the second overtone and first combination band region used for the analysis in the FT-NIR spectrometer. Thus, the same Ge detector may be used to measure the Raman and absorbance spectra of the sample. Initial combination spectra were acquired on the hybrid instrument. For the first analysis of MTBE in gasoline samples, prediction errors of 0.5 % relative by mass were obtained. Work in progress includes the determination of water in the gasohol SRMs, assignment of uncertainties to individual ampoules, and assistance in the certification of a synthetic fuel SRM using FT-Raman/NIR techniques.

5. Chemometric/Statistical Evaluations of DNA Analysis Techniques for Human Identification

D.L. Duewer, M.K. Kline (Biotechnology Division), and D.J. Reeder (Biotechnology Division)

Objective: To characterize the statistical properties of restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) measurement techniques as used in routine human identity casework practice. Knowledge of the within- and among-laboratory measurement characteristics is critical for efficient inter-jurisdictional exchange of information.

Problem: "DNA Fingerprinting" evidence was first introduced as evidence in a criminal trial in 1987. Today, over 100 municipal, county, state, federal, and private forensic laboratories routinely characterize samples with one or more DNA measurement technologies. Matching evidential and known-felon data collected in different jurisdictions requires quantitative knowledge of "how good" the measurements among laboratories really are. Knowing *why* measurements differ among laboratories facilitates technology improvement. NIST is an active member of the Technical Working Group for DNA Analysis Methods (TWGDAM), with the explicit challenge of supplying appropriate Standard Reference Materials, technology evaluations, experimental designs, and data analyses.

Approach: DNA technologies are rapidly evolving, requiring characterization of (1) casework practice of established techniques, (2) interlaboratory robustness of "coming on-line" techniques and materials, and (3) the intrinsic limitations of proposed materials and methodologies. In collaboration with the Federal Bureau of Investigation and various TWGDAM laboratories, we combine retrospective analysis of routine control data with results from prospective multi-laboratory studies and experiments using our own analytical resources.

Results and Future Plans: The interlaboratory reproducibility of RFLP measurements has been quantitatively described. We have used this quantitative understanding to document that the observed small differences between measurements made on pristine and weathered samples are not measurement artifacts but reflect changes in the electrophoretic mobility of the DNA itself. We have developed quality control charts for use within forensic laboratories and for multi-laboratory performance evaluations.

PCR technologies are replacing the sample-intensive RFLP techniques. We have shown that the apparent electrophoretic size of these DNA fragments depends on experimental parameters. A multi-laboratory study of one commercially available PCR kit confirmed our results. All study participants returned appropriate qualitative results relative to known alleles, but in the absence of standard allelic calibrants the quantitative results were unacceptably variable.

We are completing our characterization of RFLP measurements and incorporating this knowledge into the statistical procedures used to evaluate "DNA Fingerprint" comparisons. We are currently designing experiments for validation of multiplex PCR systems.

Publications:

Duewer, D.L., Currie, L.A., Reeder, D.J., Leigh, S.D., Liu, H.-K., and Mudd, JL, **"Interlaboratory Comparison of Autoradiographic DNA Profiling Measurements. 2.Measurement Uncertainty and Its Propagation,"** Anal. Chem. 67 (7), 1220-1231 (1995).

Stolorow, A.M., Duewer, D.L., Reeder, D.J., Buel, E., and Herrin, Jr., G., **"Interlaboratory Comparison of Autoradiographic DNA Profiling Measurements. 3. Repeatability and Reproducibility of RFLP Band Sizing, Particularly Bands of Molecular Size >10k Base Pairs,"** Anal. Chem. 68 (11), 1941-1947 (1996).

Kline, M.C., Redman, J.W., Reeder, and D.J., Duewer, D.L., **"Intercomparison of DNA Sizing Ladders in Electrophoretic Separation Matrices and Their Potential for Accurate Typing of the D1S80 Locus,"** Appl. Theor. Elect. 6, 33-41 (1996).

6. Investigation of Systematic Bias in Karl Fischer Amperometric and Coulometric Methods of Moisture Measurement

S.A. Margolis

Objective: To assess the accuracy of the Karl Fischer method for the determination of moisture in oil and develop reference materials to provide accuracy benchmarks for such measurements.

Problem: Electrical transformers use refined crude oils as insulation fluids. Moisture accumulates in the fluid over time and becomes critical at levels approaching 50 μg/g oil. Accurate moisture measurements are essential in the range of 10 μg/g to 50 μg/g to monitor the initial and ongoing suitability of the oil for transformer use. Accurate measurements are also economically important for fuel oil and specialty chemical companies because the water content of these costly materials can adversely affect their value. Research conducted at NIST over the past two years identified a major source of bias in the method most frequently used for determining trace levels of moisture in transformer oils.

Approach: Karl Fischer titration is the method of choice for determining low levels of moisture in liquids. It is based on a reaction of an organic iodide complex with water to release free iodide. The amount of water is determined by measuring either the amount of iodide that must be oxidized (coulometric method) or the volume of iodine that must be added (volumetric method) to reach a preset electrochemical potential. With the coulometric Karl Fischer instrumentation, the iodide produced by the reaction with water is electrochemically oxidized to reach a preset null point. This approach provides a direct measure of moisture content and does not require external calibration if the null point is properly set. Alternatively, the amount of water released can be determined by volumetric addition of iodine with moisture content determined through use of a calibration curve. Most commercial testing laboratories use the coulometric Karl Fischer approach.

To assess the bias between the two methods, parameters such as titration vessel solvents, oil solubility, multiphase systems, choice and accuracy of standards, and instrumental sources of systematic bias were evaluated. Four different oils were used to assess the effect of oil composition on the titration process in a collaborative study involving several laboratories and instrument systems.

Results and Future Plans: NIST laboratory research and interlaboratory studies showed that coulometric and volumetric Karl Fischer measurements of trace levels of moisture in petroleum-based oils differ by as much as 50 % relative. The major source of bias appears to be the solvents/solvent blends used with the coulometric Karl Fischer instruments. Chosen for their electrochemical properties, these solvents do not completely dissolve the oil matrix to allow total release of the water. The highest results were observed from volumetric measurements in the presence of chloroform; lower values resulted when the oil was not completely dissolved in the titration vessel solvent, when the chloroform level was reduced, or when the coulometric method was used. We demonstrated that the preset values of some coulometric instruments were improperly chosen resulting in a negative bias of 5 % to 20 % relative.

Based on these studies the American Society for Testing and Materials (ASTM) Committee D-27 is discussing the reformulation of the Karl Fischer solvent mixtures to ensure complete dissolution of petroleum-based oils prior to analysis. It has been proposed that the ASTM method for moisture in oils be amended to incorporate the use of NIST Moisture-in-Oil standards (RMs 8506 and 8507) for measurement quality assurance.

Future plans include: (1) optimizing the composition of coulometric solvents and calibration methods, (2) investigating the role of oil composition and other nonpolar additives and surfactants, and (3) evaluating the use of differential scanning calorimetry for the measurement of moisture in oils in the 10 µg/g to 50 µg/g range.

Publication:

Margolis, S., **"Amperometric Measurement of Moisture in Transformer Oil Using Karl Fischer Reagents,"** Anal. Chem. 67, 4239-4246 (1995).

7. Optical Wedge Effects in Instruments and Standards for Molecular Absorption Spectrophotometry

J.C. Travis and M.V. Smith

Objective: To provide for the production and accurate calibration of Standard Reference Materials for verifying the measurement accuracy and calibrating the wavelength scale of molecular absorption spectrometers or spectrophotometers. The program is under continuous review and revision to accommodate changing needs and to improve both the accuracy and utility of the standards.

Problem: Recently it has become necessary to modify the optical wedge tolerance of solid filter standards to accommodate a relatively new commercial spectrophotometer design that is destined to become an important sector of the installed instrument base, especially for real-time, on-line monitoring applications. NIST was alerted that the optical wedge, or angle between the entry and exit faces, of individual solid NIST filter standards was sufficient to cause bias in the indicated absorbance reading in some multichannel instruments. This effect is caused by a slight deflection of the optical beam, shifting the image of the source lamp on the entry slit of the wavelength-dispersive spectrometer.

Approach: The problem has been approached on several fronts simultaneously, including theoretical modeling of the relationship between wedge and deflection, negotiating improved grinding and polishing services, and development of the technology within the Analytical Chemistry Division to check the optical wedge individually on every filter produced.

Results and Future Plans: Investigation of the origins of this effect in sample beam transfer optics and in normal filter construction has led to the tightening of filter specifications and production quality control, as well as leading to some potentially important observations regarding normal cuvette usage, diagnostic measures, and instrument design considerations.

A collimator/telescope arrangement was assembled to measure the optical wedge in the filters based on the same principle that was causing the spectrophotometric bias. With a resolution of about 5 seconds of arc, the instrument is used to reject filters demonstrating an optical wedge in excess of 20 seconds of arc. In its present configuration, more than one filter per minute may be processed. Early problems with operator fatigue were alleviated with the use of a small solid-state video camera and monitor.

A test batch of 40 filters was obtained from the manufacturer of the "neutral gray glasses" used for these filters. None of the test filters showed measurable wedge, a very hopeful sign for future production.

The NIST optical shops will continue to grind the quartz for our metal-on-quartz filter standards. However, they have assembled a diagnostic instrument similar to ours and will be testing filters during production.

A manuscript is in preparation to describe the calculations involved in assessing the potential spectrophotometric bias and calibrating the wedge measurement. The information should be of use to instrument designers and to end users interested in techniques to avoid and/or detect wedge-related bias.

8. *Characterization of Thin Films for High Technology Application Using Cold Neutron Depth Profiling*

G.P. Lamaze, H.H. Chen-Mayer, and J.K. Langland

Objective: To provide elemental concentration and profile information for light elements in thin films used in industrial applications.

Problem: Manufacturers of high-technology devices need to know both thickness and composition of surface layers on their devices. Two recent investigations have included nitrogen concentration measurements in TiN for the Intel Corporation and lithium profile measurements in multilayers for SAGE Electrochromics. The latter measurements were particularly challenging in that the lithium mobility was being assessed in active devices.

Approach: Both of these measurements presented challenges for the cold neutron depth profiling instrument. Depth profiling of nitrogen is based on the measurements of the proton from the $^{14}N(n,p)^{14}C$ reaction. The nitrogen measurements were the first quantitative evaluation of nitrogen concentrations since the instrument was revised and placed on the curved neutron guide (see technical report #12). The advantages of this new setup were apparent in the higher signal rate and the lower background rate compared with nitrogen measurements made at the previous instrument location.

The lithium profiles are based on the measurement of the energy of alpha particles from the $^{6}Li(n,\alpha)^{3}H$ reaction. The energy of the detected particle provides a direct measurement of the depth of the originating lithium nucleus. In this case, *in situ* measurements were taken with different bias voltages on the film layers. The bias causes the lithium to migrate between different layers which changes the optical transparency of the film.

Results and Future Plans: Figure 1 shows the nitrogen profiles obtained for the thinnest of the titanium nitride films. The manufacturer was able to correlate these results with the conditions of the film making process. Future plans call for increasing resolution and further reducing the background. This will allow us to measure films that are both thinner and of lower nitrogen concentration.

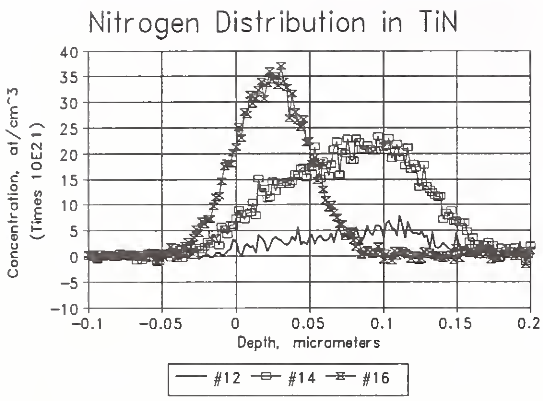


Figure 1. Nitrogen profile of three different TiN samples. Negative depth values are due to the system resolution

Figure 2 shows the lithium profile of an electrochromic multilayer under two different voltage conditions as well as the resulting difference spectrum. The cross-hatched areas represent the total amount of lithium moved by the voltage change. Future plans call for making simultaneous optical transmission measurements and lithium profile measurements.

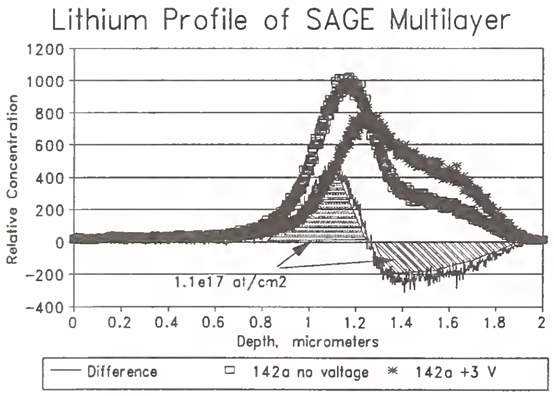


Figure 2. Lithium profile at two different voltages with difference spectrum

Publication:

Lamaze, G.P., Chen-Mayer, H., Langland, J.K., and Downing, R.G., **"Neutron Depth Profiling with the New NIST Cold Neutron Source,"** Surf. & Int. Anal. (in press).

9. Determination of Uniformity, Purity, and Homogeneity of Prototype TiN Thin Films on Silicon

P.A. Pella, M. Lankosz, J. Pedulla, and R.D. Deslattes (Atomic Physics Division, PL)

Objective: To develop reference materials consisting of thin films of various thicknesses of titanium nitride on silicon substrates.

Problem: X-ray fluorescence spectrometry is often used in the semiconductor industry to monitor the composition, areal density, and uniformity of thin films on various substrates during the fabrication operation. For calibration of the measurement process, thin films of the same type having known composition and thickness are required. Currently there are no NIST reference materials to benchmark measurement accuracy in such applications. There are many types of films of different composition and thickness that the semiconductor industry needs for calibration purposes. Meetings between NIST and representatives of the semiconductor industry (SEMATECH) resulted in the decision that reference materials consisting of titanium nitride films on silicon substrates should be given highest priority. This SRM is being developed in cooperation with the Atomic Physics Division.

Approach: Prototype films of titanium nitride on silicon wafer substrates (7.6 cm diameter) with nominal thicknesses of 100 nm were prepared by ion-beam sputtering from a commercial TiN target using an argon beam. The fabrication of the material and characterization of the film thickness, material density, and interfacial surface roughness were carried out by the Quantum Metrology Group using x-ray reflectivity spectrometry. The uniformity, purity, homogeneity, and stoichiometry were measured by the Spectrochemical Methods Group using x-ray fluorescence spectrometry.

Results and Future Plans: X-ray fluorescence measurements indicated that the purity of the films was quite acceptable. Aside from argon, the only contaminants detected (iron, nickel, and chromium) were introduced from the stainless steel sputtering chamber. X-ray analysis of titanium in the TiN film showed that the stoichiometry was within 1% relative to the theoretical stoichiometry of 1:1. This was confirmed by Rutherford backscattering measurements. A TiN coated wafer will be subdivided into nominal 2 cm x 2 cm platelets to provide the actual samples for the user community. Therefore, it is important to maximize the film uniformity over most of the area of a 7.6-cm wafer. This will allow an optimum number of samples to be obtained per wafer and minimize the fabrication time. The uniformity and homogeneity of the film deposit were measured by x-ray microfluorescence spectrometry by step scanning a 1 mm diameter x-ray beam across the diameter of the wafer. As shown in Figure 1, the film thickness near the edges of the wafer is about 5 % less than that nearer the center.

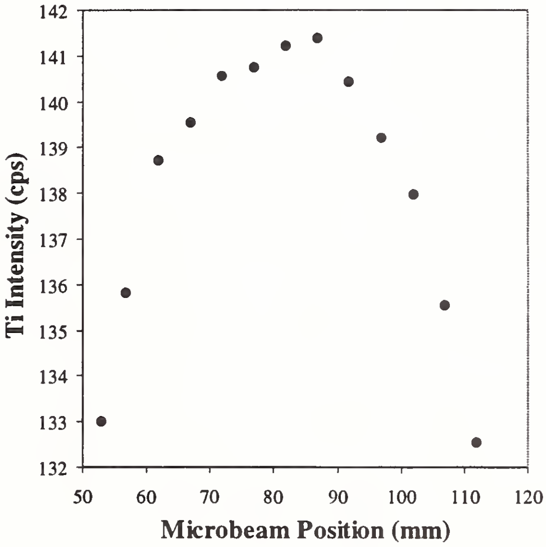


Figure 1. X-Ray microfluorescence scan showing variation of TiN film thickness across the diameter of a 7.6 cm silicon wafer

We found that TiN films could be prepared having the required purity, homogeneity, stoichiometry, and thickness to produce a TiN SRM. We plan to improve the uniformity of the film deposition across the entire wafer by using a planetary substrate holder that will allow independent rotation of the wafer during the sputtering operation. After preparing sufficiently uniform films, we plan to fabricate a master set for final characterization. Upon successful characterization of the master set, we plan to prepare the final reference material.

10. Determination of Sulfur in Aerospace Superalloys Near the Limit of Quantitation by Isotope Dilution Thermal Ionization Mass Spectrometry

W.R. Kelly, R.D. Vocke, Jr., and T.L. Quinn

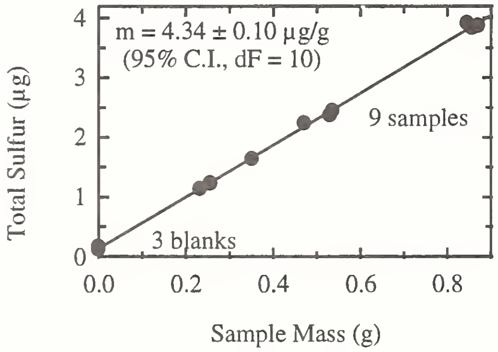
Objective: To provide support to the U.S. aerospace industry's need to measure accurately the sulfur content in superalloys at 1 µg/g and below.

Problem: The U.S. aerospace industry is the leader in R&D spending on new technologies and is the nation's leading net exporter of manufactured goods. The most critical components of jet engines are the so-called "hot parts" with the high pressure (HP) turbine being one of the most critical. The inlet temperature to the HP turbine determines the upper limit on the power and efficiency of a turbine-based power source. Turbine blades are cast using high temperature superalloys that are high in Ni with moderate amounts of Cr and Co, and smaller amounts of very refractory elements such as Re. The adherence of a protective oxide layer to the surface of turbine blades is adversely affected by sulfur concentrations greater than 1 µg/g, but the problem disappears at levels below about 0.2 µg/g. Thus, the industry must measure sulfur accurately at these levels. The protective oxide layer is also important for the next generation of turbine blades that will be made by applying a ceramic thermal barrier coating to this oxide surface. There are currently two problems associated with low sulfur measurements: 1) there are no superalloy standards certified for sulfur, and 2) the current instrumentation is operating near its detection limit.

Approach: The determination of sulfur as the AsS^+ molecular ion by isotope dilution thermal ionization mass spectrometry (ID-TIMS) is currently the only method with the required accuracy to address this problem. However, sulfur measurements on sub-gram samples at the 1 µg/g level and below are near the limit of quantitation (blank ≅ analyte). The variability of the analytical blank is currently the fundamental limitation to accuracy. The arsenic sulfide procedure uses a highly enriched ^{34}S spike for quantitation. It has been used to measure the sulfur concentration in SRM 131e (Low Alloy Steel) and several superalloys. Following sample and blank combustion, the equilibrated sulfur in the sample is reduced to H_2S and then precipitated as As_2S_3. The $^{75}As^{32}S^+$ and $^{75}As^{34}S^+$ ion ratios are measured in a NIST built TIMS. The measurement reproducibility (2s) is typically 0.2 %, relative, which is much smaller than all other sources of uncertainty. The blank problem is minimized by using a least squares approach in which blank samples are measured together with samples of differing masses.

Results and Future Plans: SRM 131e, the only standard currently used by the aerospace industry for low sulfur measurements, was recently re-certified using this least squares approach. The resulting certified value of 4.34 µg/g ± 0.10 µg/g expanded uncertainty has a much lower uncertainty than the 1991 consensus-based certified value of 4 µg/g ± 2 µg/g (± 2s). The data used for the recertification of SRM 131e are shown in Figure 1. The fact that the blanks are consistent with the intercept predicted by the actual samples indicates that the blank measurement is a valid measurement of contamination levels in the actual samples. Similar results have also been obtained for S in several superalloys below the 1 µg/g level. This same approach will be used to certify a superalloy SRM.

Figure 1. Least squares analysis of samples and blanks for the determination of sulfur in SRM 131e



11. *Measurement of k_0 Factors for Prompt Gamma Activation Analysis*

R.L. Paul

Objective: To establish a database of k_0 factors to facilitate calibration-free measurements by prompt gamma-ray activation analysis (PGAA).

Problem: Because neutron scattering and neutron absorption in PGAA targets give rise to analytical bias, element sensitivities (counts s^{-1} mg^{-1}) are highly dependent upon both target matrix (mainly hydrogen content) and geometry. Analytical uncertainties arising from these processes may be minimized by careful matching of sample and standard matrix and geometry. However, this is both time-consuming and tedious if new standards must be prepared for each new matrix analyzed. An alternate approach is to apply the method of k_0 factors to PGAA.

Approach: The k_0 method of quantitation, used extensively in delayed neutron activation analysis, makes use of the fact that ratios of two measured quantities can be measured more accurately than the individual quantities. The k_0 factor is a ratio calculated using the sensitivities of both the target and the comparator elements and the detection efficiencies of the gamma-rays measured, and is independent of the neutron energy, the sample matrix and geometry, or the counting geometry or detector used. For a given PGAA facility, it may be shown that the ratio of the sensitivity of an element to that of a comparator element is independent of neutron energy or sample matrix and geometry. Accurate measurements of element sensitivities and detector efficiencies as a function of energy may be used to generate k_0 factors that may be used by all PGAA facilities. The use of k_0 factors allows for accurate multielemental analysis without the need for multielement standards.

Results and Future Plans: Measurements were made at the NIST reactor using both the cold neutron PGAA instrument located in the Cold Neutron Research Facility and the University of Maryland-NIST thermal neutron PGAA instrument. Element sensitivities were determined from analysis of standards of pure materials; detector efficiencies were determined as a function of gamma-ray energy using calibrated radioactive standards. The data were then used to calculate k_0 factors for PGAA. As predicted by theory, k_0 factors calculated from cold and thermal neutron data are in agreement within the measured uncertainties.

Future efforts will be devoted to developing better methods for fitting the efficiency/energy data to reduce uncertainties in the measured k_0 values. Accurately measured k_0 factors for a large number of elements will then be compiled into a database and made available to PGAA facilities worldwide.

Publication:

Paul, R. L., "**The Use of Element Ratios to Eliminate Analytical Bias in Cold Neutron Prompt Gamma-ray Activation Analysis,**" J. Radioanal. Nucl. Chem. 191, 245-256 (1995).

12. Recent Activities in Cold Neutron Focusing

***H.H. Chen-Mayer**, D.F.R. Mildner, and V.A. Sharov (X-Ray Optical Systems, Inc.)*

Objective: To increase both the elemental sensitivities and the spatial resolution for Prompt Gamma Activation Analysis (PGAA) and Neutron Depth Profiling (NDP) at the NIST Cold Neutron Research Facility.

Problem: PGAA and NDP provide important chemical compositional information for a wide variety of high-tech materials. However, a higher neutron intensity is required to measure small samples accurately. In addition, compositional mapping information is often desired. PGAA currently provides only bulk information, and NDP only depth information. A higher intensity neutron beam, focused on a small spot, will allow PGAA and NDP to provide two- and three-dimensional compositional mapping information, which is extremely useful for materials characterization.

Approach: The best method of focusing a white beam of long wavelength neutrons is based on multiple mirror reflection from smooth surfaces. The long wavelengths from the macroguide enter the narrow cylindrical guides of polycapillary glass fibers and are transported by successive grazing angle reflection. A lens contains over a thousand fibers of diameter of 0.5 mm, each with over a thousand hollow capillaries of diameter 10 μm. The fibers are curved such that the neutron trajectories are directed towards a common focus, where the sample under investigation is placed.

Results and Future Plans: Our polycapillary fiber lens has been placed on the end of one of the guides for PGAA measurements. The lens compresses a neutron beam of cross section 50 mm x 45 mm and a current density of 6.5×10^8 cm^{-2}s^{-1} onto a focal spot of diameter 0.53 mm (FWHM) at a distance of 52 mm from the exit of the lens, and with an average gain in neutron current density by a factor of 80. PGAA measurements have demonstrated a gain in gamma count rate of 60, a gain in signal-to-noise of 7, and a 20-fold improvement in detection limits. These increases are obtained despite the fact that only 3.5 % of the neutron beam incident on the lens is delivered to the focus. Consequently, there is need both to increase the lens efficiency and, more importantly, to reduce the background from (n,γ) reactions from the unfocused neutrons.

Further improvements in the signal-to-noise ratio will result in further reductions in the detection limits. In collaboration with X-Ray Optical Systems, we have designed a lens that not only focuses the beam 100 mm from the exit of the lens, but also bends the beam downward by 42.5 mm, out of the direct path of the incident beam. The transmission characteristics have been analyzed using a photostimulable luminescent imaging plate placed at the focal plane to measure the spatial intensity distribution. Our initial measurements have shown a decrease in the background and a focal spot of 0.65 mm (FWHM), but the complex curvatures of the polycapillary fibers have allowed only a gain of about 20 in the neutron current density at the focus. However, the lower gain may be compensated by the lower background to achieve a better detection limit. Efforts are underway to reduce the background even further.

The background from unfocused neutrons can be reduced and efficiency increased by increasing the acceptance area at the entrance of the lens. This can be achieved in angular space by the use of metal capillaries with critical angles larger than glass, and in real space by the use of tapered capillaries. Work is underway on both of these approaches.

A monolithic lens consisting of a fused tapered bundle of polycapillaries has been shown to provide a smaller focus of about 0.16 mm, and gains comparable to that of the polycapillary lenses. The physical dimensions of these devices are much smaller than for the polycapillary lenses, so they are useful for the NDP instrument with its more constrained space. The goal is to perform three dimensional compositional mapping of thin-film semiconductor materials using NDP with the monolithic tapered lens to achieve greater lateral resolution and higher sensitivity.

Publication:

Chen-Mayer, H., Sharov, V.A., Mildner, D.F.R., Downing, R.G., Paul, R.L., Lindstrom, R.M., Zeissler, C.J., and Xiao, Q.F. ***"Capillary Neutron Optics for Prompt Gamma Activation Analysis,"*** J. Radioanal. Nucl. Chem. (in press).

13. Developments in High-Accuracy Wavelength Measurements Using UV/Visible Fourier Transform Spectroscopy

M.L. Salit and J.C. Travis

Objective: To evaluate the limit of precision and characterize the uncertainties associated with wavelength measurements using UV-visible Fourier-transform spectra.

Problem: Discrepancies in the Fourier transform spectroscopy (FTS) of wavelength standards in Ar and ^{198}Hg raised questions regarding the consistency of the FTS wavelength scale. Our work investigating these discrepancies demonstrated a level of consistency of about a part in 10^8 in the wavelength scale, prompting an effort to measure new wavelength standards from these two species. We have been refining our measurements to eliminate sources of uncertainty, and to establish appropriate uncertainty bounds to report for the standards.

Approach: The system used to characterize the discrepancies is also an unprecedented system for making broadband wavelength measurements, previously limited to accuracy of about 1 part in 10^7. By simultaneously observing two optical frequencies, one of whose frequencies is accurately known and whose relationship is exactly known, we can both calibrate a spectrum and test the uniformity of the wavelength scale. A laser and a harmonic of that laser (where the harmonic frequency is an exact multiple of the fundamental), with one of the frequencies stabilized to a well-known absorption transition, is a practical realization of such a source. Employing an integrating sphere to combine this calibration light source with the ^{198}Hg source ensures spatially homogenous illumination of the FTS entrance aperture, assuring accurate calibration. The appearance of the narrow and stable lines from the calibration source is a sensitive measure of instrument line shape (ILS), allowing a useful diagnostic for optical alignment — critical to eliminating uncertainty arising from asymmetric lines.

Results and Future Plans: Using the two-color frequency standard (which has uncertainties on the order of a part in 10^9) as a calibrant, we have been able to make measurements of the strongest lines in ^{198}Hg with standard deviation of the mean of 1×10^{-9} of the wavelength or smaller. A bias that must be accommodated in our uncertainty estimate arises from the deviation from the expected frequency ratio of the two-color standard. This appears to be on the order of 7 parts in 10^9, with scatter as high as 1 part in 10^8. Figure 1 presents results for the ^{198}Hg "green" line, and the accepted value for the wavelength of this line, with its uncertainty.

In initial experiments with this system we observed unexpected trends in the apparent scale bias, and we have successfully taken steps to eliminate potential sources for this drift. Using the ILS as an alignment diagnostic, we developed and implemented optical alignment schemes that have yielded a symmetric ILS. Using this system, the measurements of the new ^{198}Hg line standards will be accomplished in FY 1997 by measuring the brightest lines in the integrating sphere with the two-color standard and calibrating the weaker lines with these bright lines directly coupled to the FTS.

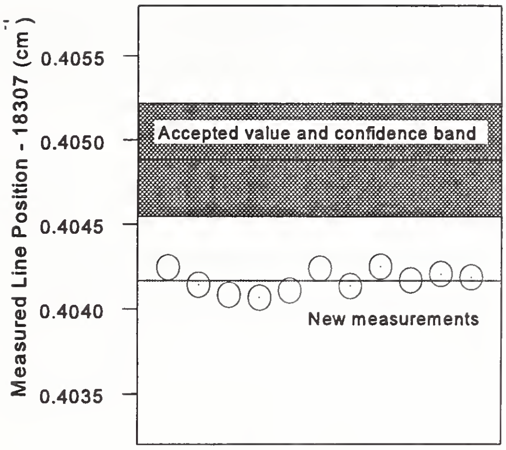


Figure 1. Measurements of 546.1 nm line position in ^{198}*Hg and accepted standard*

Publication:

Salit, M.L., Travis, J.C., and Winchester, M.R., **"Practical Wavelength Calibration Considerations for UV-visible Fourier Transform Spectroscopy,"** Appl. Optics 35, 2960-2970 (1996).

14. On-Line Coulometry for Process Applications

K.W. Pratt

Objective: To design and evaluate a continuous on-line coulometric analyzer for process streams.

Problem: Existing on-line process analyzers require chemical calibration using a standard calibrant solution or a prestandardized solution that is continuously consumed during operation. In either case, traceability of the measured result relies on the accuracy and long-term stability of the calibrant or standard solution. In addition, many instrumental sensors require that the analyzer be taken off-line for calibration, increasing down time and reducing process throughput.

Approach: An analyzer that functions according to Faraday's Laws of Electrolysis would be an absolute technique; i.e., one in which chemical calibration is *not* required. In the system under development, the titrimetric reactant is generated coulometrically in a titrant flow stream that is separate from the sample (process) stream. These two streams mix downstream from the coulometric cell and then pass through an endpoint detector. The signal from the endpoint detector governs the coulometric generation current via a feedback loop. At steady state, the output of the coulometric generator equals the flux of analyte in the sample stream. Separate generation has the advantage that the electrolyte and electrodes used for the coulometric titrant generation can be chosen to assure 100 % current efficiency, without concern for interfering components of the process stream. The gain and time response of the feedback loop are critical to the correct functioning of the system. The flow rate, v_r, of the *titrant* stream and the response time, τ, of the endpoint detector determine the net response time of the feedback loop: large v_r and small τ yield optimal performance.

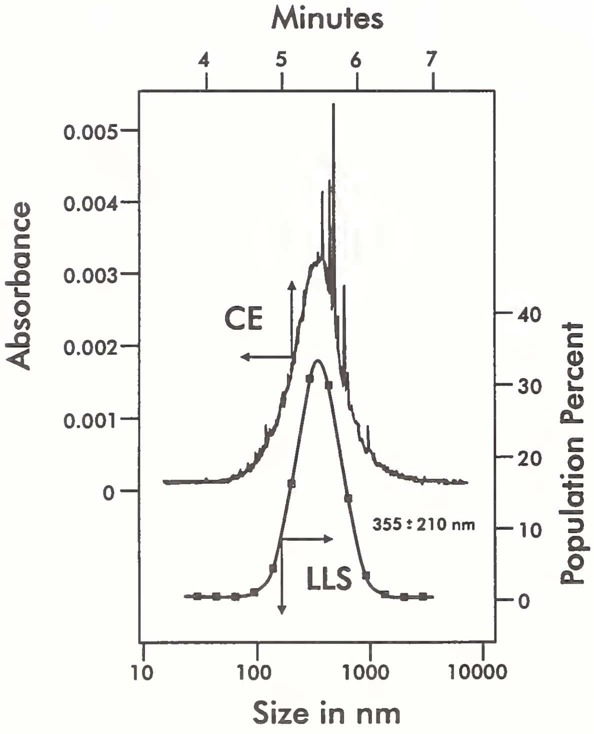
Results and Future Plans: Results have verified the fundamental theory for dilute solutions of strong acids (0 to 3 mmol/L H^+). Typical system relative uncertainty is ±0.5 %, sufficient for projected process applications. System time to obtain 90 % of the theoretical response value is typically 3 min with the ISFET (ion-selective field-effect transistor) sensor. Concentrated solutions require an accurate means of diluting the sample solution (a low-dead-volume, continuous diluter). Applicability to weak acids has been demonstrated and optimization is currently underway.

Additional work is expected to demonstrate feasibility to basimetric titrations including NH_4OH. Work will be initiated for on-line coulometry of Cr(VI) process streams. Work is also in progress to increase the stability of the feedback loop. Two anticipated applications to real processes are the real-time determination of ammonium hydroxide used in etching baths for Si wafers and the determination of Cr(VI) in chromate plating baths.

15. *Characterization of Liposomes in Capillary Electrophoresis (CE)*

__W.A. MacCrehan__, L.E. Locascio, L.E. Martynova, M.A. Roberts (Cornell University), and R.A. Durst (Cornell University)

Objective: To develop a new tool for characterizing liposome preparations.



Problem: In aqueous solution, phospholipids self-assemble to form a lipid bilayer membrane surrounding an aqueous cavity, forming a spherical structure known as a liposome. The composition of both the outer lipid bilayer and the internal solution may be tailored to act as a molecular "Trojan Horse" providing a powerful approach to the selective delivery of active agents for cosmetic, pharmaceutical, and analytical applications. Liposomes are being used for such diverse applications as delivering moisturizers to the skin, targeting anticancer drugs to tumors, and for the amplification of immunogenic events for a flow bioassay. Both the lipid composition and preparation protocol can markedly affect the chemical and physical properties of the liposomes. The vesicle size, effective charge and stability may be altered, affecting the suitability for their intended application. To date, liposomes have been primarily characterized by laser light scattering, which provides physical information on the average diameter of the liposomes but no information on the chemical properties or amount of material present.

Approach: In a collaborative project with Cornell University, we evaluated the use of CE as a new tool to characterize and quantitate liposome preparations. We developed an understanding of liposome behavior in the analytical system. Liposome interactions with the capillary wall were characterized and a rinsing/analysis protocol was developed that permitted reproducible measurement of CE peak area and migration time. We then applied CE to determine liposome solution behavior.

Results and Future Plans: The distribution of the liposome population determined by CE and laser light scattering were in reasonable agreement; see Figure. The relationship of concentration and peak area was linear over a 300-fold range. Within-day and between-day measurement reproducibility were determined. The results demonstrated that CE can provide quality control data for monitoring liposome production processes.

Electrophoretic mobility was used to calculate the net charge on each liposome. We also used CE to study the processes controlling detergent lysis of the liposomes. The ability of the liposome bilayer to partition hydrophobic molecules was also shown by the migration of riboflavin through a zone containing liposomes. Partitioning increased the riboflavin migration time two-fold, suggesting the use of liposomes as pseudo-stationary phases for analytical separations.

We are investigating application of liposome/CE for the detection of biomolecules, using bilayer-membrane-immobilized antibodies/receptors as analyte-recognition elements. CE is being used as a powerful test-bed for the development of selective chemistries suitable for application in microchannel sensor devices, which we are currently fabricating.

Publication:

Roberts, M.A., Locascio, L.E., MacCrehan, W.A., and Durst, R. A., **"Liposome Behavior in Capillary Electrophoresis,"** Anal. Chem. 68, 3434-3440 (1996).

16. Development of Techniques for the Structural Characterization of Monoclonal Antibodies

D.M. Bunk

Objective: To develop techniques for the structural characterization of monoclonal antibodies using liquid chromatography (LC) coupled with electrospray ionization mass spectrometry (ESI-MS).

Problem: With the increasing reliance on immunoassay for clinical diagnostic testing, it is important to obtain as much information as possible about the monoclonal antibodies that serve as the principal functional element of these immunoassays. During the development of an immunoassay, the binding strength and specificity of the binding of the monoclonal antibody to its target molecule, the antigen, are thoroughly evaluated. However, little structural characterization of the antibody is performed. Structural diversity of an antibody used in an immunoassay can have a pronounced effect on the outcome of the assay, limiting its accuracy and the soundness of any ensuing clinical diagnosis. The reason for this limited degree of structural characterization is that there are no techniques available that can readily provide information on antibody chemical structures. So far, mass spectrometry has been applied only to a limited degree to the characterization of antibodies, but has the promise to provide fast, detailed, and accurate structural information.

Approach: Antibodies are very large biomolecules ($M_r \approx 150{,}000$) with complex structures, e.g., multiple crosslinked protein chains, attached carbohydrates, variations in amino acid sequence, etc. This complexity limits the information provided by mass spectrometry on intact antibodies. Fortunately, there are a number of established procedures for antibody fragmentation that produce fragments small enough to be amenable to mass spectrometric analysis. These procedures include enzymatic fragmentation, enzymatic deglycosylation, and affinity chromatographic methods. By combining these fragmentation procedures with mass spectrometry, the fragments of the antibodies can be thoroughly characterized. In particular, ESI-MS is the ideal mass analysis technique because of its ability to be coupled to separation techniques such as LC.

Results and Future Plans: The initial stage of this research project has been aimed at improving the separations that could be obtained for antibody fragment digests, in particular, separations that can be directly coupled to mass spectrometry. Differences in liquid chromatographic column chemistries (C_4, C_8, C_{18}, and diphenyl columns) and the influence of temperature were studied. Increasing the temperature had a dramatic effect on the quality of the chromatographic separation of the digest samples. At room temperature, LC/ESI-MS analysis of an antigen binding fragment indicated that it was homogeneous, but analysis at 60 °C revealed substantial heterogeneity in the fragment structure. Further work is proposed to couple ion-exchange chromatography to mass spectrometry using in-line microdialysis to remove salts from the mobile phase to reduce interference to the mass spectrometry.

Analysis of the fragmentation digests of mouse IgG monoclonal antibodies revealed distinct molecular mass patterns of the F_c fragment from different mouse antibody isotypes. With additional study, this research could lead to a rapid and accurate method for isotyping antibodies. More detailed structural characterization of the F_c fragments is needed to determine which structures contribute to the different isotype molecular mass patterns that are observed.

17. Column Coupling Techniques in Supercritical Fluid Chromatography for the Separation of Chiral Compounds

K.L. Williams, L.C. Sander, and S.A. Wise

Objective: To demonstrate the use of coupled achiral/chiral columns in supercritical fluid chromatography for the separation of structurally related chiral compounds.

Problem: Although liquid chromatography (LC) incorporating chiral stationary phases (CSPs) can resolve many enantiomers, the poor achiral selectivity of most CSPs limits separation of the enantiomers of interest from structurally related compounds such as impurities or metabolites. As a result, two separate analytical methods are often needed to determine the achiral and chiral purities of the sample. Multi-column techniques have been developed in LC to address this limitation. However, coupling of achiral and chiral columns in LC, either directly or through column switching devices, is often restricted by incompatibility of the mobile phases for the chiral and achiral stationary phases.

Approach: Carbon dioxide modified with a small amount of methanol has been used as an eluent for a variety of chiral and achiral stationary phases in supercritical fluid chromatography (SFC). Columns can be coupled in series to modify selectivity and increase the number of theoretical plates without pressure drop limitations or problems of mobile phase incompatibility. As long as the stationary phases are stable under the chromatographic conditions used in SFC, virtually any combination of columns is possible. Therefore, inexpensive achiral stationary phases can be used to modify the selectivity of the chromatographic system without compromising the enantioselectivity of the CSP. Because retention and selectivity characteristics of achiral stationary phases also tend to be more predictable than those of CSPs, molecular structure can be used to guide selection of the appropriate achiral stationary phase. SFC has the additional advantages of high efficiency, rapid method development, and reduced analysis times when compared to LC for chiral separations. Groups of structurally related compounds chosen for investigation of the coupled-column approach included calcium channel blockers, β-blockers, and 1,4-benzodiazepines. A CSP based on 3,5-dimethylphenylcarbamoylated cellulose (Chiralcel OD) was utilized as the chiral component of the coupled column system. Carbon dioxide modified with either methanol or ethanol was used as the eluent. Chromatographic conditions were chosen based on optimum conditions for the CSP.

Results and Future Plans: In the absence of an achiral stationary phase, chromatographic analysis of mixtures of structurally similar compounds such as β-blockers on the Chiralcel OD CSP suffered from peak overlap in SFC. Although the compounds were enantioresolved, the enantiomers of different compounds were not well resolved from each other. When an achiral column was coupled in series with the CSP, separation of all the sample components was dramatically improved and co-elution was eliminated. Successful applications of achiral/chiral column coupling were demonstrated using amino, cyano, and diol bonded phases as the achiral component of the coupled column system. No change in chromatographic conditions was required after addition of the achiral stationary phase. The column-coupling technique provides a mechanism to streamline the chiral method development process and should reduce the need for separate assays to determine achiral and chiral purity. This technique should also prove useful for the analysis of the growing number of pharmaceutical compounds having multiple chiral centers.

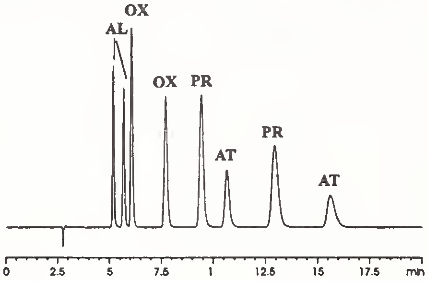


Figure 1. Enantioseparation of four chiral beta-blockers using the coupled achiral/chiral column system

Publication:

Williams, K.L., Sander, L.C., and Wise, S.A., "**Comparison of Liquid and Supercritical Fluid Chromatography for the Separation of Enantiomers on Chiral Stationary Phases.**" J. Pharm. Biomed. Anal. (in press).

18. *Low-Level Light Standards for Chemiluminescence and Other Photon-Counting Detectors*

G.W. ***Kramer****, J.F. Morrison, and J.C. Travis*

Objective: To assess and prioritize industry needs for low-level and single-photon-counting light intensity standards for clinical and analytical luminescence measurements; to identify one or more promising standards technologies and investigate the parameters that will need to be controlled to make candidate technologies viable for use as standards.

Problem: Luminescence-based assays have become very popular in clinical diagnostics because of the low detection limits and wide dynamic range that can be achieved with relatively simple instrumentation. These assays ultimately measure the light produced as a result of chemical (chemiluminescence, CL) or biological (bioluminescence, BL) reactions. Typically, CL or BL reagents are employed as labels to tag molecules for quantitative detection. With potential detection limits down to attomolar (10^{-18} mol/L) levels, CL and BL tags are now rapidly replacing fluorescent and radiochemical labels in many clinical, forensic, chemical, and biochemical test methods.

Despite the increasing popularity of luminescent techniques, there are currently no convenient, traceable standards for absolute light intensity to compare, calibrate, verify linearity, or determine wavelength responsivity of the low-level light and photon-counting detectors used in these assays.

Approach: In response to numerous requests from the clinical diagnostics manufacturing community to address the lack of standards, in FY 1996 we held a workshop with manufacturers and users of low-level light measuring instruments to plan a national program to develop standards and a standards traceability scheme for low-level light and single-photon-counting measurements. The workshop was organized jointly by the Chemical Sensing and Automation Technology group in the Analytical Chemistry Division and the Optical Temperature and Sources and Optical Materials and Infrared Technology groups in the Optical Technology Division.

Results and Future Plans: Approximately 35 scientists and engineers from the clinical diagnostics community (both manufacturers and end-users) attended the NIST Workshop on Low-Level Light Standards for Luminometry on May 1, 1996. Discussions at the workshop delimited the needs and useful ranges of functional parameters for potential standards such as intensity levels, wavelengths, area intensity uniformity, accuracy and uncertainty levels, short- and long-term stability, useful life, and temperature range. Two types of "gold" standards are desired to meet the needs of both manufacturers and end-users of low-level light instrumentation and diagnostic reagent kits. Instrument manufacturers need *artifact standards* (low-level sources with defined geometry) that are traceable to SI units for the factory calibration of their instrumentation, while manufacturers of clinical diagnostic test kits and end users want *chemical standards* (convenient, reproducible reactions with known quantum yields) that are linked to the CL or BL chemistries employed in their assays. To address both of these needs, a two-phase program was proposed involving 1) the development of a national reference instrument at NIST and an artifact transfer standard traceable to the NIST instrument for use primarily by instrument manufacturers to calibrate detectors, and 2) research into and evaluation of suitable luminescent chemistries to determine the feasibility of developing "field" calibration standards that could be employed by diagnostic kit manufacturers and end users to test instruments and/or the entire assay procedure.

Work during the next fiscal year will be aimed at identifying suitable candidate technologies for artifact standards and investigating the parameters that will need to be controlled to make these technologies viable for use as standards. We have begun to build a research luminometer for use as a "survey instrument" to study candidate standards. Initially, we will investigate electrically controllable intensity standards such as light emitting diodes (LEDs), since the workshop attendees indicated a strong preference for this type of device as an artifact standard. LED devices and electronic drive techniques will be examined with respect to spectral distribution of light intensity, temperature effects, device stability and uniformity of light distribution patterns, and spatial distribution of light output.

19. Development of a Glass Wavelength Standard for Near Infrared Spectrophotometry

J.C. Travis and S.J. Choquette

Objective: To develop a glass wavelength standard for industrial manufacturers and users of near infrared (NIR) spectrophotometers.

Problem: NIR spectroscopy is becoming a mainstay in chemical process measurements with applications in petrochemical, pharmaceutical, agricultural, and health industries. However, NIST has no optical filter reference materials for the NIR spectral region (~700 nm to 2500 nm). Because of the nature of the spectra obtained in NIR measurements, multivariate statistical methods are commonly needed to perform an analysis. To ensure the accuracy of the results, the wavelength axis of NIR spectrometers must be calibrated frequently. Although polymer-film consensus standards exist, several thicknesses of these materials must be used to cover the complete NIR spectrum. As a result, NIST has started a program to develop a rare earth oxide glass that may be used throughout the entire NIR spectral region.

Approach: In 1993, a particular composition of rare earth oxides in glass was identified, that yields well-defined peaks over the spectral region corresponding to the popular InGaAs detector, 700 nm to 1700 nm. Samples of this glass were cut and polished into 1 mm thick, 25 mm diameter optics. Forty of these filters are currently under study using a Fourier Transform-NIR spectrophotometer to acquire the NIR absorbance spectrum of each filter. Peak stability, temperature coefficients of the peak position, and sample homogeneity are being evaluated for these filters. Since the user community has specified a 0.1 cm^{-1} absolute peak position uncertainty, several peak-picking algorithms are currently under study to attain these levels of precision.

Results and Future Plans: A variable-window cubic polynomial curve fit was used to evaluate the peak positions of these filters. Currently, our best efforts yield only two identifiable peaks that yield the desired 0.1 cm^{-1} peak position reproducibility over the 40 filters studied. However, an alternative peak picking algorithm, the center of gravity method (COG), yields six peaks with the required reproducibility. Unfortunately, the COG method is more difficult to use and sometimes gives peak positions that are slightly different than the apparent peak maximum. As a result, the user community is not enthusiastic about incorporation of a COG method in their validation measurements. Future work will focus on optimizing the variable-window polynomial fit to increase the number of certifiable peaks and perhaps modifying the COG method to reduce the apparent peak shifting.

20. Development of Food- and Nutrition-Related SRMs

***K.E. Sharpless**, D.A. Becker, J. Brown Thomas, R. Demiralp, P.M. Ellerbe, R.R. Greenberg, E.A. Mackey, B.R. Norman, L.T. Sniegoski, M.J. Welch, S.A. Wise, L.M. Gill (Statistical Engineering Division, ITL), and S.B. Schiller (Statistical Engineering Division, ITL)*

Objective: To certify several natural matrix Standard Reference Materials (SRMs) for selected vitamins, carotenoids, fatty acids, cholesterol, trace elements, and other nutrients.

Problem: The Nutrition Labeling and Education Act (Public Law 96-359) requires that nutritional information be provided for all packaged foods sold in the U.S. after May 1994. In addition, the Infant Formula Act (Public Law 101-535) requires that specific nutrients contained in infant formula fall within a specified range or above a specified range or above a specified minimum. Food-matrix SRMs are needed by the entire food testing/nutritional laboratory community including commercial food industry, university research, government regulatory agency, and consumer group laboratories. Food-matrix SRMs are needed to facilitate compliance with nutritional formulation requirements and labeling laws; to provide traceability for food exports needed for acceptance in many foreign markets; and to assist in the provision of accurate labeling information to assist consumers in making sound nutritional choices.

Results and Future Plans: NIST is actively working to provide an increased array of SRMs certified for such important nutrients as vitamins, cholesterol, fatty acids, and minerals as well as for toxic trace elements. Nutrient concentration values were assigned to two such SRMs in 1996: SRM 1544, Fatty Acids and Cholesterol in Frozen Food Composite; and SRM 1846, Infant Formula. SRM 1544, a frozen food composite developed in cooperation with the U.S. Department of Agriculture (USDA), is intended for laboratories involved in the analysis of foods for individual fatty acids and cholesterol. Certified values are provided for six fatty acids based on gas chromatography/mass spectrometry (GC/MS) measurements from NIST and GC measurements at three outside laboratories. Cholesterol values were determined using the NIST definitive method, and the proximates (protein, carbohydrate, fat, etc.) were certified based on measurements from three outside laboratories. SRM 1846, Infant Formula, was developed in cooperation with the USDA, the U.S. Food and Drug Administration, and the Infant Formula Council in response to the requirements of the Infant Formula Act of 1980. Value assignment of proximate and caloric content as well as the concentrations of 9 minerals and trace elements; and 16 vitamins was based on NIST measurements of selected vitamins and the results from nine outside laboratories.

NIST is currently working on two additional SRMs for selected nutrients: SRM 1548a, Typical Diet, and SRM 2383, Baby Food Composite. SRM 1548a, Typical Diet, will be a replacement for SRM 1548, Total Diet, and was prepared in conjunction with the USDA. It is a freeze-dried composite of a large variety of foods representing the total daily diet consumed by an individual in the U.S. This SRM will be certified for a number of dietary components including proximates, major and minor inorganic nutrients, and toxic trace elements. Certification will utilize results from outside laboratories as well as NIST measurements. Another food composite, SRM 2383, is a material with high levels of selected vitamins and carotenoids. It was prepared and packaged by Gerber Products Company using a NIST-specified mixture of individual food components. Certification will be based on values from NIST, from an interlaboratory exercise involving 20 member laboratories of the National Food Processors Association, and from an interlaboratory comparison involving 12 additional outside laboratories. In FY 1997 we will begin work on a meat homogenate SRM certified for cholesterol, fat as specific fatty acids, minerals, and proximates.

21. Quality Assurance Activities in Support of Health-Related Measurements

M.J. Welch, J. Brown Thomas, P.M. Chu, D.L. Duewer, S.A. Margolis, M.S. Rearick, K.E. Sharpless, L.T. Sniegoski, P.M. Ellerbe (College of American Pathologists), S.E. Long (College of American Pathologists), S. Tai (College of American Pathologists), and W.J. Thorn III

Objective: To provide measurement quality assurance (QA) support for laboratories performing health-related measurements.

Problem: Inaccuracy in health-related measurements increases overall health-care costs, results in misdiagnoses, and leads to inaccurate conclusions in clinical studies. Health-care costs represent over 13 % of the U.S. GNP and have been increasing at twice the rate of our national economy in recent years. Measurements represent about 20 % of the cost of health care, and approximately one-third of these measurements are performed for non-diagnostic purposes, such as retesting, error prevention, and detection limitations.

Approach: In a long-term relationship with NIST, the College of American Pathologists (CAP) sponsors three research associates at NIST to work with NIST scientists on method development and reference materials to support accuracy in clinical analyses. The CAP operates a large proficiency testing program for clinical laboratories. Analyses performed at NIST using isotope dilution mass spectrometric (ID/MS) definitive methods are used to evaluate the accuracy of the field methods.

NIST also works closely with the National Cancer Institute to provide measurement QA for over 60 laboratories conducting analyses of serum levels of potential cancer chemopreventive agents in clinical trials. Interlaboratory studies, reference materials, and workshops are used to document and improve the accuracy of the measurements of a number of important micronutrients. Method development activities for new compounds that are identified as potential anticancer agents are part of the program.

Results and Future Plans: Three new frozen serum SRMs were issued that provide the clinical laboratory community with a direct traceability link to NIST definitive and reference methods. For SRM 1951a (Lipids in Frozen Human Serum) ID/MS measurements at NIST were used to certify cholesterol and triglycerides. In addition, noncertified values were provided by the Centers for Disease Control and Prevention, using its reference methods — for total, HDL-, and LDL-cholesterol and for triglycerides. For SRM 956a (Electrolytes in Frozen Human Serum) four electrolytes — calcium, potassium, magnesium, and lithium — were certified by ID/MS and sodium was determined by the NIST definitive gravimetric method. SRM 965 (Glucose in Frozen Human Serum) was certified using the NIST ID/MS definitive method for glucose and that method will be used to regularly monitor the material to determine if storage at -80 °C is sufficient to prevent changes in the glucose concentration over time. A number of CAP materials were also value-assigned using NIST measurement methods, thus providing additional tools to laboratories for establishing traceability to NIST. NIST efforts coupled with those of industry and other government agencies have resulted in marked improvements in clinical laboratory accuracy and precision for many important health markers.

Similar improvements have been observed for laboratories participating in our QA program for micronutrients in blood, serum, and food. Studies have shown that the improved inter- and intra-laboratory reproducibility can be attributed to the information, reference materials, and technical consultations provided by this QA program.

Future plans include continued CAP-NIST cooperation in developing new measurement methods and providing NIST-traceable reference materials. During the coming year, a new CAP reference material for sweat chloride, a marker for cystic fibrosis, will be value-assigned at NIST. A new ID/MS method for serum thyroxine (a thyroid function marker) will be completed and work will begin on ID/MS methods for serum cortisol (an endocrine function marker) and toxic trace metals in blood.

Interlaboratory comparison exercises and an annual QA workshop will continue to be important components of the micronutrient QA effort. Method development activities will continue, with emphasis on the flavonoids, a class of compounds that are found in many foods, have significant antioxidant properties, and thus likely anticancer activity.

In FY 1997, NIST support for health-related measurements will also include the development and critical evaluation of methods for the determination of alpha-fetoprotein in serum, speciated iron in serum, and low level cadmium in whole blood as well as refinement of the NIST definitive method for glucose. We will also develop gas mixture standards and a new prototype facility for evaluating NO/NO_2 sensors to support the development of a new therapeutic regimen for pulmonary disorders in newborns.

Publications:

Margolis, S.A. and Duewer, D.L., "**Measurement of Ascorbic Acid in Human Plasma and Serum: Stability, Intralaboratory Repeatability, and Interlaboratory Reproducibility,**" Clinical Chemistry 42, 1257-1262 (1996).

Duewer, D.L., Thomas, J. Brown, Kline, M.C., MacCrehan, W.A., Schaffer, R., Sharpless, K.E., May, W.E., and Crowell, J.A., "**NIST/NCI Micronutrients Measurement Quality Assurance Program: Measurement Reproducibility, Repeatability, Stability, and Relative Accuracy for Fat-Soluble Vitamin-Related Compounds in Human Sera,**" Anal. Chem. (in press).

22. Quality Assurance Activities in Support of Environmental Measurements

*S.A. **Wise**, E.A. Beary, R. Demiralp, M.K. Donais, T.L. Green, R.R. Greenberg, F.R. Guenther, M. Lopez de Alda-Villaizan, E.A. Mackey, A.F. Marlow, J.R. Moody, K.E. Murphy, R.M. Parris, P.A. Pella, B.J. Porter, D.L. Poster, M.S. Rearick, L.C. Sander, M.M. Schantz, J. Smeller, G.C. Turk, T.W. Vetter, and L.J. Wood*

Objective: To provide measurement quality assurance (QA) support for laboratories performing environmental measurements.

Problem: Decision-making based on inaccurate measurements or data of unknown quality can have significant economic or health consequences. Natural-matrix SRMs representative of different types of environmental samples and intercomparison studies are needed to assist in the validation of analytical methods and for the assessment and documentation of the quality of environmental analytical measurements.

Approach: To address the above-mentioned needs, we have been involved in the development and certification of several environmental matrix SRMs during the past year: (1) organic, inorganic, and organometal constituents in marine sediment and tissue matrices; and (2) lead in various household matrices. Two certification modes were used for these SRMs. The marine environmental SRMs were certified using various combinations of NIST and outside laboratory measurements. The lead reference materials were certified based solely on NIST measurements.

NIST also provides other activities in support of several national environmental monitoring activities. In the past year, we initiated a new QA activity in conjunction with the Environmental Protection Agency (EPA) and the Aluminum Manufacturers' Association to assist in the monitoring of gases that may contribute to global warming. These gases are emitted from aluminum processing plants when the extraction process has become inefficient. Thus, it is in their economic best interest, as well as in the interest of reducing global warming, that the processing plants reduce the emissions. Since 1986 we have worked in cooperation with the National Oceanic and Atmospheric Administration (NOAA) and the EPA to assess the interlaboratory and temporal comparability of data for organic contaminants from laboratories involved in the NOAA National Status and Trends (NS&T) Marine Monitoring Program and the EPA Environmental Monitoring and Assessment Program (EMAP). In 1993 we initiated a quality assurance activity in support of the EPA National Health Exposure Assessment Survey, which is intended to provide critical information about the magnitude, extent, and causes of human exposures to environmental contaminants so that informed decisions can be made regarding environmental health risks. In the above programs, NIST provides QA support, including the development of needed SRMs and other control materials, coordination of interlaboratory comparison exercises, evaluation of the interlaboratory data, analysis of sample splits with the participating laboratories, and coordination of workshops to discuss the results of these activities.

Results and Future Plans: For organometal speciation needs, we applied a recently developed method based on measurements by gas chromatography with atomic emission detection (GC-AED) for the determination of methylmercury in three mussel tissue SRMs: SRM 1974a, Organics in Mussel Tissue (*Mytilus edulis*) (Frozen); SRM 2974, Organics in Freeze-Dried Mussel Tissue; and SRM 2976, Trace Elements in Mussel Tissue. The methylmercury results from the GC-AED method were combined with results from two other laboratories, which used analytical methods that were independent from the NIST method, to provide certified methylmercury values for all three mussel materials. These three materials are the first natural-matrix environmental SRMs with certified metal speciation values, and they are the first mussel tissue matrix certified reference materials (CRMs) certified for methylmercury content. SRM 2976 also represents the lowest certified level of methylmercury in any existing CRM. In addition to methylmercury values as described above, SRM 2974, which is a freeze-dried material prepared from the same mussel tissue homogenate as SRM 1974a, has certified values for polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated pesticides. Both SRM 1974a and SRM 2976 will have certified and reference values for selected trace elements based on measurements at NIST using instrumental neutron activation analysis and results from eight other laboratories that participated in an interlaboratory comparison exercise. We recently completed the certification analyses for a new sediment material, SRM 1944, a contaminated marine sediment, which will have certified values for PAHs, PCBs, chlorinated pesticides, and trace elements at levels approximately 10 times higher than previous marine sediment SRMs.

Progress continued during the past year on the development of new reference materials, in conjunction with EPA, the Research Triangle Institute, and the U.S. Geological Survey, designed for use in the assessment of lead contamination. The full suite of new SRMs includes: three powdered paints with different lead concentrations (SRMs 2581, 2582, 2589), two indoor dust samples with different levels of lead contamination (SRMs 2583 and 2584), two soils that have been contaminated by lead paint (SRMs 2586 and 2587), paint films on Mylar sheets and fiberboard designed for the calibration of portable x-ray analyzers (SRM 2579 and RM 8680), and a simulated bone reference material designed as a standard for *in-vivo* methods for measuring lead in bone. Analytical work has been completed for most of these materials, and several have already been issued. The full suite of lead SRMs will be completed in the coming year.

The results from the global-warming gases program and the marine monitoring program illustrate the impact of NIST interlaboratory comparison activities. For the EPA/Aluminum Manufacturers' Association project, NIST provides standards of the global-warming gases for calibration of the analytical methods and provides well-characterized process stream samples for use in interlaboratory comparison exercises. There are two aluminum extraction processes that give different emissions levels. The first interlaboratory comparison results, on a sample from a "Prebake" cell process, demonstrated that the participating laboratories are all producing results within 20 % of the NIST value. In the next year a second interlaboratory comparison sample, which is representative of the "Soderberg" cell process, will be collected and distributed to the participating laboratories. For the NOAA NS&T and EPA EMAP QA program, NIST efforts focus on improving the comparability of measurements for over 60 organic contaminants in marine bivalve tissue, sediment, and fish samples. Current participants in these interlaboratory exercises represent a number of multi-laboratory monitoring programs and include over 40 federal, state/municipal, university, private sector, and international laboratories. At the beginning of this activity, the program managers had no tools to assess the comparability and quality of the measurements made by the various laboratories providing data for their monitoring programs, nor could they effectively document changes in the quality of the data produced. With continuous participation in these annual intercomparison exercises, laboratories can now demonstrate the quality and comparability of their results.

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23. *Establishing a National Marine Analytical Quality Assurance Program*

S.A. Wise, P.R. Becker, and B.J. Porter

Objective: To establish a National Marine Analytical Quality Assurance Program that will include interlaboratory comparisons, reference materials development, and environmental specimen banking.

Problem: Inaccuracies in marine monitoring measurements can have significant economic or health consequences. Improvements in accuracy and intra- and inter-laboratory measurement reproducibility enhance detection of small but significant trends in contaminant levels, enable informed decision-making based on fewer measurements, and facilitate leveraged use of data produced by different laboratories for different studies. Tools such as reference materials and interlaboratory comparisons are required to evaluate, improve, and document the quality of these analytical measurements. A national program is needed to expand NIST interactions with the U.S. marine environmental community in order to strategically assess and prioritize measurement needs, foster collaborative efforts, and expand the scope of existing NIST interlaboratory comparison, reference material, and specimen banking activities.

Approach: NIST and the National Oceanic and Atmospheric Administration (NOAA) have collaborated in the area of marine environmental quality assurance for more than 10 years. In 1995, NIST and NOAA National Marine Fisheries Service (NMFS) signed a Memorandum of Understanding to formalize this collaboration and to develop a National Marine Analytical Quality Assurance Program. The purpose of this program is: (1) to assess and improve the quality of analytical measurements in the marine environment through interlaboratory comparisons and reference material development; and (2) to improve the capabilities to assess trends in marine environmental quality by expanding specimen banking activities. These purposes will be accomplished by the following:

- Collaboration/consultation to identify quality assurance, reference materials, and specimen banking needs in the marine environment;
- Establishment of a specimen bank facility to focus on marine environment samples. This facility will expand NIST capabilities for retrospective analyses of the marine environment;
- Production of control materials, proficiency testing materials, and reference materials that are representative of marine matrices for use in the evaluation of analytical measurement quality;
- Cooperation in the preparation and certification of NIST SRMs for marine environmental measurements;
- Education of scientists relative to quality assurance and specimen banking procedures, e.g., conduct training sessions and host workshops/symposia.

Results and Future Plans: We are currently working with the NOAA NMFS Southeast Fisheries Science Center in Charleston to establish a new research facility to be called the "Marine Environmental Health Research Laboratory" (MEHRL). The MEHRL will be a premier high-technology marine research center encouraging collaborative efforts among scientists at federal, state, and academic institutions to apply new scientific techniques to fisheries and marine resource management issues. NIST will have a major presence at MEHRL including an environmental specimen bank, reference material development/production facilities, and state-of-the-art analytical laboratories. NIST is renovating existing laboratory space in the Charleston Laboratory to provide specimen banking and analytical laboratory facilities until the MEHRL is completed in 2000 (target date). At present one NIST scientist is located at the NMFS laboratory to implement and coordinate NIST activities there. NIST staffing levels will increase as the program grows and the new facility is completed.

Publication:

Becker, P.R., Wise, S.A., Thorsteinson, L., Koster, B.J., and Rowles, T., **"Specimen Banking of Marine Organisms in the United States: Current Status and Long-Term Prospective,"** Chemosphere (in press).

24. NORAMET Chemical Measurement Intercomparisons

W.E. May, S.A. Wise, and R.R. Greenberg

Objective: To establish comparability of chemical measurements among the three national metrology laboratories within North American Metrology Cooperation (NORAMET): National Institute of Standards and Technology (NIST) in the United States, National Research Council (NRC) of Canada, and Centro Nacional de Metrologia (CENAM) in Mexico.

Problem: Accurate and reliable chemical measurement data are becoming increasingly important. Chemical measurement data are used daily in the conduct of international trade, in environmental and health decision-making, and in assessing product performance and safety. However, until recently, little thought was given to the establishment of any formal system for assuring the international comparability of chemical measurements. The past several years have witnessed the establishment of regional metrological groups for North America, South America, the European Union and the Asian-Pacific Rim. Although the current activities within the various groups differ somewhat at this time, they are all working toward the harmonization of chemical measurements within their respective spheres of influence and on various protocols for evaluating and assuring harmony among the various regional metrology groups. The chemical measurement comparability activities for the North American continent are spearheaded by the Chemical Metrology Committee within NORAMET.

Approach: During the past two years, representatives from NIST, NRC-Canada, and CENAM met on two occasions to formulate a program for establishing chemical measurement comparability within North America. The initial meeting was held on May 5, 1995 at NIST and the second meeting was held in Ottawa on October 15, 1996 at NRC-Canada. Plans were established for regular intercomparisons among the three National metrology laboratories, collaborations on reference materials development projects, the establishment of a NORAMET Chemical Metrology homepage on the World Wide Web and the convening of a "Chemical Measurement Traceability Symposium" at NIST in the spring of 1998.

Results and Future Plans: CENAM was established in 1994 as Mexico's central Metrology Laboratory and during the past 2½ years, 14 CENAM scientists have worked in the Analytical Chemistry Division for a combined total of approximately 360 weeks observing and learning NIST approaches to chemical measurements and the certification of reference materials. During this same time period, Division scientists have spent 35 staff-weeks providing consultations at CENAM. During the coming year plans are to collaborate on:

- Recertification of a sucrose SRM
- Development of a primary standards suite for natural gas
- Characterization of Al and Cu alloys
- Marine environmental analysis
- Automated fusions for x-ray fluorescence spectrometry
- Nutrients in foods

During the past year, NIST and NRC-Canada compared results from the determination of several trace metals (As, Cr, Mn, Sb, Sr, and Zn) and 14 polychlorinated biphenyl (PCB) congeners in marine tissue and sediment samples. The only significant bias observed for the trace metals (at concentrations ranging from 10 µg/g to 1000 µg/g on a dry basis) was for As, where the NIST values were consistently slightly higher. NIST measurements were made using Instrumental Neutron Activation Analysis and the NRC-Canada measurements were made using Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The small difference may be attributed to either incomplete dissolution or loss during the dissolution process that preceded GFAAS. Preliminary review of the PCB results shows reasonable comparability for all 14 congeners (at concentrations ranging from 2 ng/g to 100 ng/g dry basis in the marine tissue). Biases range from 0 % to 25 % relative. Investigations are underway to identify the sources of the differences observed.

The intercomparisons will be expanded in FY 1997 to include all three NORAMET partners and will focus on trace metals in drinking water and trace metals, chlorinated pesticides, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons in marine sediments and tissue.

25. Intercomparison of NIST Primary Standards with European "Gateway Laboratories"

F. R. Guenther, J.R. Moody, P.A. Berezansky, W.D. Dorko, and W.E. May

Objective: To provide a horizontal comparability link between NIST and the European Union through intercomparisons of primary standards.

Problem: In many cases international trade is impacted by the measurement of important chemical species. These measurements must be made in accordance with the receiving country's regulations, and these regulations usually demand traceability to national standards. This may represent a barrier to trade unless more sources for traceable standards are available. By building horizontal comparability links, either the source or receiving country's national standards can be used. Another benefit of these intercomparisons is the identification of bias in primary standards maintained by the collaborative parties. This results in more accurate and precise primary standards for both countries.

Approach: In the area of primary standard gas mixtures (PSM), a Memorandum of Cooperation (MoC) was signed on July 1, 1994 with the Netherlands Measurements Institute (NMi) that outlines a program of intercomparisons for all PSMs in common between the two Institutes. Each year a new Declaration of Equivalence that lists the PSMs that are equivalent is agreed to and signed. In previous years four PSMs (carbon dioxide, carbon monoxide, ethanol, and propane) have been shown to be equivalent.

This year we established a new MoC among NIST, the Danish Institute of Fundamental Measures (DIFM), and the Hungarian National Office of Measures (OMH) for the measurement of electrolytic conductivity, which is of importance and economic significance to manufacturing process control, pharmaceutical and semiconductor manufacture, and power generation. This agreement will document the comparability of electrolytic conductivity measurements among these laboratories and also between NIST and European Union laboratories.

Results and Future Plans: During the past year under the MoC with NMi, three additional PSMs were shown to be equivalent. We have resolved a bias that was discovered in the sulfur dioxide PSMs, and the two Institutes now agree to within 0.05 % over the entire concentration range. We have also shown agreement on the nitric oxide and oxygen PSM suites. However, there is a 2 % bias in the hydrogen sulfide PSMs that is currently under active investigation.

Under the terms of the new MoC, for electrolytic conductivity, samples are to be initially exchanged between the laboratories once per year. The first exchange of samples was accomplished in the summer of 1996. The results from the OMH and DIFM have been received at NIST and are being statistically evaluated, along with results of the measurements performed by NIST. The first exchange involved a nominal 0.1 S/m solution. Future solutions to be measured include 0.01 S/m and 1.0 S/m solutions. The allowable bias on the measurements has been agreed not to exceed 0.3 % for the 0.01 S/m solution, and 0.2 % for the other two solutions.

An MoC on pH is being actively pursued with the European Union for implementation within the next year.