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NBS TECHNICAL NOTE 544

Analytical Coordination Chemistry Section:

Summary of Activities
July 1969 to June 1970

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Summary of Activities July 1969 to June 1970

O. Menis and J. I. Shultz, Editors

Analytical Coordination Chemistry Section
Analytical Chemistry Division
Institute for Materials Research
National Bureau of Standards
Washington, D.C. 20234

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FOREWORD

The Analytical Chemistry Division was established as a separate division at the National Bureau of Standards on September 1, 1963, and became part of the Institute for Materials Research in the February 1, 1964 reorganization. It consists at present of nine sections and about 100 technical personnel encompassing some 60 different analytical competences from activation analysis and atomic absorption to vacuum fusion and x-ray spectroscopy. These competences, and in turn the sections which they comprise, are charged with research at the forefront of analysis as well as awareness of the practical sample, be it standard reference material or service analysis. In addition it is their responsibility to inform others of their efforts.

Formal publication in scientific periodicals is a highly important output of our laboratories. In addition, however, it has been our experience that informal, annual summaries of progress describing efforts of the past year can be very valuable in disseminating information about our programs. A word is perhaps in order about the philosophy of these yearly progress reports. In any research program a large amount of information is obtained and techniques developed which never find their way into the literature. This includes the "negative results" which are so disappointing and unspectacular but which can often save others considerable work. Of importance also are the numerous small items which are often explored in a few days and which are not important enough to warrant publication--yet can be of great interest and use to specialists in a given area. Finally there are the experimental techniques and procedures, the designs and modifications of equipment, etc., which often require months to perfect and yet all too often must be covered in only a line or two of a journal article.

Thus our progress reports endeavor to present this information which we have struggled to obtain and which we feel might be of some help to others. Certain areas which it appears will not be treated fully in regular publications, are considered in some detail here. Other results which are being written up for publication in the journal literature are covered in a much more abbreviated form.

At the National Bureau of Standards publications such as these fit logically into the category of a Technical Note. We plan to issue these summaries for all of our sections. The following is the sixth annual report on progress of the Analytical Coordination Chemistry Section.

W. Wayne Meinke, Chief
Analytical Chemistry Division

PREFACE

The sixth annual progress report of the Analytical Coordination Chemistry Section describes the research and development activities which, as in previous years, have been directed toward the needs and projections of the Standard Reference Materials Program. This year, for example, witnessed the reissuance, after thirty-five years, of two clay standards. The best methods presently available for the determination of many of the constituents in these complex materials still involve classical analytical chemistry. As such, a tremendous demand is placed on today's analyst to match the high quality work of some of his predecessors. At the same time for current instrumental needs, two new standards were issued in the area of thermal analysis. Again, both absorption spectrophotometry and flame spectroscopy were employed for numerous trace determinations. One major contribution from these two competences was the determination of various elements in simulated lunar glasses with an accuracy within one percent at the parts-per-million level. Finally, as stated recently by our Division Chief, "It has been through our clinical efforts that we, as a division, have begun to appreciate the magnitude of the measurement problem in spectrophotometry." Programs are well under way for the development of standards to calibrate or check the absorbance-transmission scales for spectrophotometry. A similar program has been initiated more recently in spectrofluorimetry. All of these latter efforts are strongly backed by the clinical chemists and pathologists.

While this report is fragmentary, especially in the more recent work, it should nevertheless serve as a guide to the approaches undertaken by the Section in an attempt to solve some of the current problems. The reader's inquiries and suggestions regarding these efforts are strongly solicited.

In order to adequately describe experimental procedures, it is occasionally necessary to identify commercial products and equipment by the manufacturer's name or label. In no instances does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the particular product or equipment is necessarily the best available for that purpose.

The assistance of Mrs. Rosemary Maddock is gratefully acknowledged. Also, we acknowledge the invaluable assistance of Mrs. Mary Pantazis, Section Secretary, who performs a wide variety of duties essential to the work in this section, and for her tireless efforts in typing this report.

Oscar Menis, Chief
Analytical Coordination Chemistry

James I. Shultz, Assistant Chief
Analytical Coordination Chemistry

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ANALYTICAL COORDINATION CHEMISTRY SECTION

Summary of Activities

July 1969 to June 1970

Edited by
Oscar Menis and James I. Shultz

Progress in research and development in several areas of analytical chemistry, as related to the Standard Reference Materials program is described. Based on the need for spectrophotometric standards by clinical chemists, both solid and liquid filters for calibrating the photometric scale are being developed. Similar needs and plans are reviewed for fluorimetric quantum yield standards. Development of flame spectrometry instruments are described which include a high precision multi-element flame spectrometer for simultaneous atomic absorption and flame emission, a ratio recording double beam spectrometer, new burner designs and the evaluation of a hollow cathode designed by Grimm. The multichannel spectrometer provides for the simultaneous determination of elements such as Na, K, Ca, CaOH and Mg with Li as the internal standard, and a seventh position for background of wavelength scan, and a digital system with tape for computer calculations.

The beginning of a comprehensive study of the absorbing and non-absorbing lines in atomic absorption is presented. Description is given of homogeneity studies and the analysis of glass standards containing 60 elements at the 0.2 to 500 ppm level with a relative standard deviation of 1-2 percent. Gold, iron, manganese, nickel, potassium, rubidium and strontium were determined, without prior chemical separations by spectrophotometric or atomic absorption and flame emission methods. A precise method for the flame emission determination of aluminum in ferrous materials by derivative optical scanning is also described.

Solution complexation studies involve the reactions of metals with bilirubin and the evaluation of equilibrium constants and kinetics in solvent extraction of iron and vanadium with β-isopropyltropolone.

In the area of thermal analysis, provisional certification of potassium nitrate (SRM 756) and quartz (SRM 755) for temperature scale DTA standards, and the studies of sulfur, stearic acid and hexachloroethane for the lower temperature scale are reported. For gases in metals, improvements in inert gas fusion analysis involved instrumental modifications which permitted sample changes in less than 12 seconds. Finally, the characterization of the following Standard Reference Materials are discussed: lead-bearing alloys, SRM 53e and 1132; carbon steel, SRM 20g; stainless steel, SRM 101f; low-carbon stainless steel, SRM 166c; metallo-organic, SRM 1061c, special steels, SRM 361, 362 and 365.

Key words: Bilirubin solvent extraction with β-isopropyltropolone; clinical; filter; fluorimetric standards; inert gas fusion; lead-base alloys; multi-element flame spectrometer; spectrophotometric standards; steels and metallo-organics; thermal analysis DTA Standards; trace elements-glass standards.

1. SPECTROPHOTOMETRY

A. Introduction

A major problem which presents a challenge to the Section is the need for high accuracy spectrophotometric measurements and a shortage of suitable calibration standards. The current importance of this issue can be gleaned from statements made by scientists involved in clinical chemistry. The President of the American Association of Clinical Chemists has estimated recently [1] that, with the aid of automation, approximately one-half billion determinations on bio-clinical fluids can be anticipated this year. In a review article in the "Clinical Chemist", it was also reported by Rand [2] that over 95 percent of clinical analyses depend on spectrophotometric measurements. When one considers the fact that a large fragment of the information collected by this method is being stored in computer memory banks for future correlation with other medical data for potential use in diagnosis and genetic control, the importance of accurate data is apparent. For this reason there is an urgent need for Standard Reference Materials to calibrate not only high precision instruments, but also provide control data for an ever increasing number and variety of automated instruments. Thus the efforts of the Section are concentrated in the development of appropriate standards in spectrophotometry and spectrofluorimetry. At the same time we are developing instrumental capabilites to improve the accuracy of the measurement.

There is a need for three general types of SRM's to assure highest precision and accuracy in spectrophotometric measurements. To attain these requirements one needs all three categories. The three general types of standards are for: (1) calibrating the photometric scale, (2) calibrating the effective spectral bandpass, (3) evaluation of stray light effect.

During the past year, major attention in this Section was directed toward the first category, the development of neutral (wavelength-independent) filters for calibrating the photometric scale. Both liquid and solid optical filters have been under extensive investigation and will be described in subsequent sections of this report. This program is outlined in table 1.

Table 1. Outline of the program to develop SRMs and instrumentation for high accuracy spectrophotometry.

- Optical filters and instrumentation
  - Solids
    - Colored glass filters (neutral Corning, Chance, Schott)
    - Evaporated metals on quartz
    - Grids on quartz
    - Polarizers
    - Sectors
  - Solutions
    - Single metal in solution
    - Compound solutions
    - Organic dyes (black)
    - Mixed solutions (NBS)
  - Instrumentation
    - Design of a high accuracy single or double beam spectrophotometer

In addition to the above standards, one needs to check the bandpass limitations of the instrument. Failure to do so will lead to inaccurate molar absorptivity values when too wide a bandpass is used in measuring the absorbance of a compound with a relatively narrow absorption peak. For an example, to obtain an accuracy to within 0.1 percent the spectral bandpass must not exceed 1/20 of the natural band width of the compound under investigation. Since instruments vary in their capabilities, standards must be available for determining the effects of bandpass on the accuracy of absorbance measurements. We, therefore, proposed a program to provide standards whose absorption spectra simulate those compounds of interest to the clinical chemist. These standards, however, would be more stable and require relatively simple steps in their use.

The spectrophotometrist must be aware of and have means for measuring stray light and its adverse effect on the accuracy of his measurements. By stray light we mean any light reaching the detector that is outside the spectral region isolated by the slit. If a spectrophotometer is in adjustment, the bulk of the stray light will arise as scatter from the optics and the walls of the monochromator. Its effect, however, will depend upon the characteristics of the source and the detectors that are used. Thus, the effects of stray light are more pronounced in those regions where one of the components of the spectrophotometer is being extended to its limit of performance.

Stray light can introduce many and varied problems in spectrophotometry. In some instances spurious peaks may be observed; in others, errors may result in the measurement of absorptivity. These errors may be either positive or negative, depending upon whether the stray light is absorbed or not. It is relatively easy to understand how the presence of significant amounts of stray light can compound the absorbance errors in clinical analyses, since it is extremely difficult, if not impossible, to reproduce the matrix composition in the standards. Thus, the values reported for a particular clinical test may vary depending upon the type of interaction of the stray light with the supporting matrix. Because of this difficulty in reproducing the composition of the matrix in the standards, it is necessary that clinical chemists have some means for detecting and evaluating the stray light in their instruments. It is thus planned to select and provide appropriate standards and/or procedures for measuring instrumental stray light over the spectral region of 200-700 nm.

Other aspects covered in this chapter include developments in the determination of trace elements in simulated lunar glass SRM's.

B. Solid Materials to Check the Photometric Scale of Spectrophotometers (R. Mavrodineanu)

The use of solid filters for testing the correct functioning of spectrophotometers is a common practice. Filters for checking the accuracy of the wavelength scale, the photometric scale, and stray light in such instruments have been available to the analytical chemists for a long time. No single filter, however, can be used to provide a simultaneous control of these three instrumental parameters. Since the accurate check of the photometric scale seems to be the most critical factor, particular attention was given to the selection and study of those materials which are best suited as SRM's.

1. Colored Glass Filters

These materials should fulfill the following general conditions: have a transmittance which is independent of wavelength, exhibit a low reflection, cover a wide wavelength interval (230 to 800 nm), be stable, and readily available and inexpensive. Based on these considerations, a selection was made from the various solid transparent materials that are available. The following colored glass filters were selected: Corning-8364, Chance ON-10 and Schott NG-4.

The transmission characteristics of these filters are illustrated in figure 1.

The dependence of transmittance on the width of the spectral bandpass was then determined for the Chance ON-10 and Schott NG-4 glasses. A discussion of this important

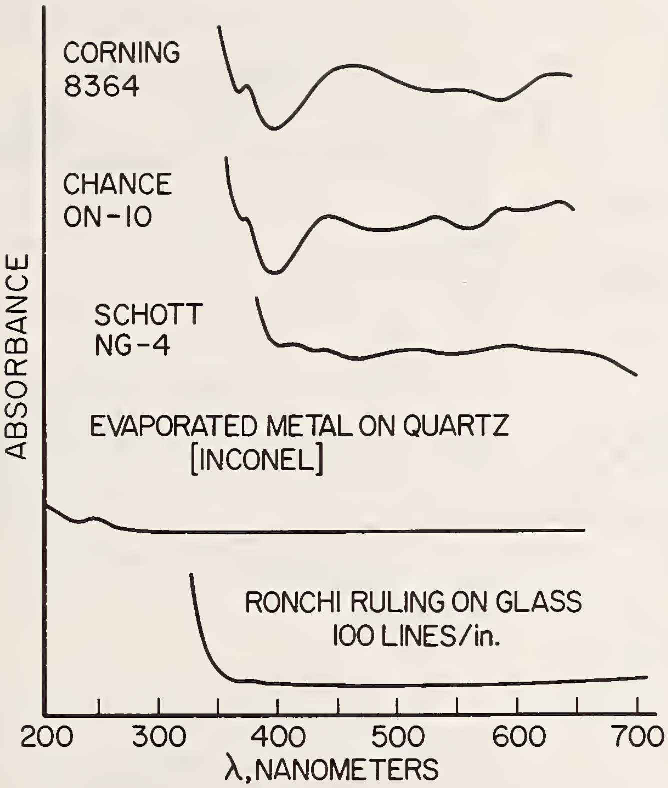

Figure 1. Optical transmittance of several selected filters.

parameter is given by Gibson [3]. For this purpose, a spectrophotometer consisting of a quartz prism double monochromator and double beam arrangement was used. The instrument is capable of producing measurements with a standard deviation for a single determination to better than 0.1 percent.

Figures 2 and 3 present the results obtained at four wavelengths. It can be seen that the Schott NG-4 colored glass filter exhibits the smallest dependence of transmittance from spectral bandpass. This property, together with the optical neutrality, illustrated in figure 1, were the determining factors in the selection of the Schott NG-4 filter as a potential SRM for the photometric scale calibration in the wavelength interval of

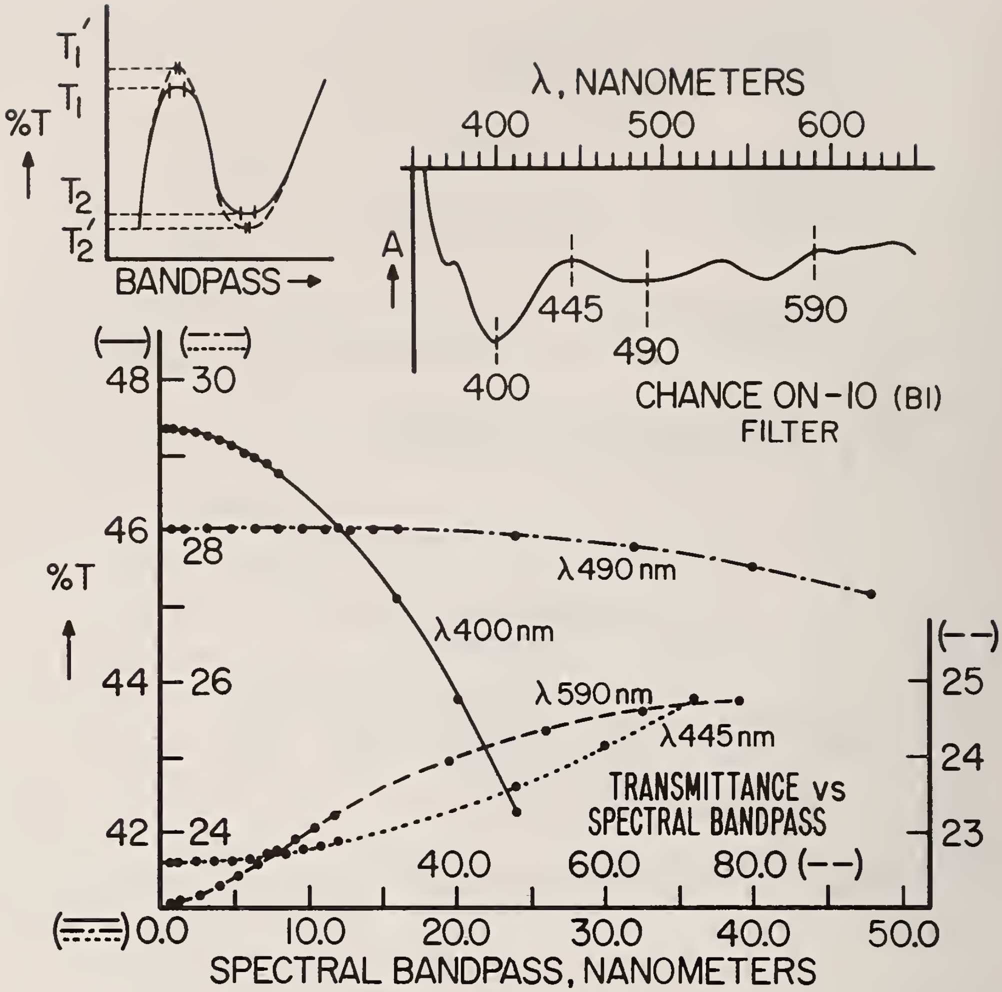

Figure 2. Optical transmittance versus spectral bandpass for a Chance ON-10 filter.

400 to 700 nm. Following these measurements, two sets of three NG-4 filters, 25 mm x 10 mm and having thicknesses of 1.0, 1.5 and 2.0 mm respectively, were prepared. Each filter was marked at one corner and placed in a metallic holder which inserts into the sample compartment of conventional spectrophotometers. The holder was marked at one side to permit positioning of the glass filter. The exposed surface of the filter, when placed in the holder, was about 8 mm x 22 mm.

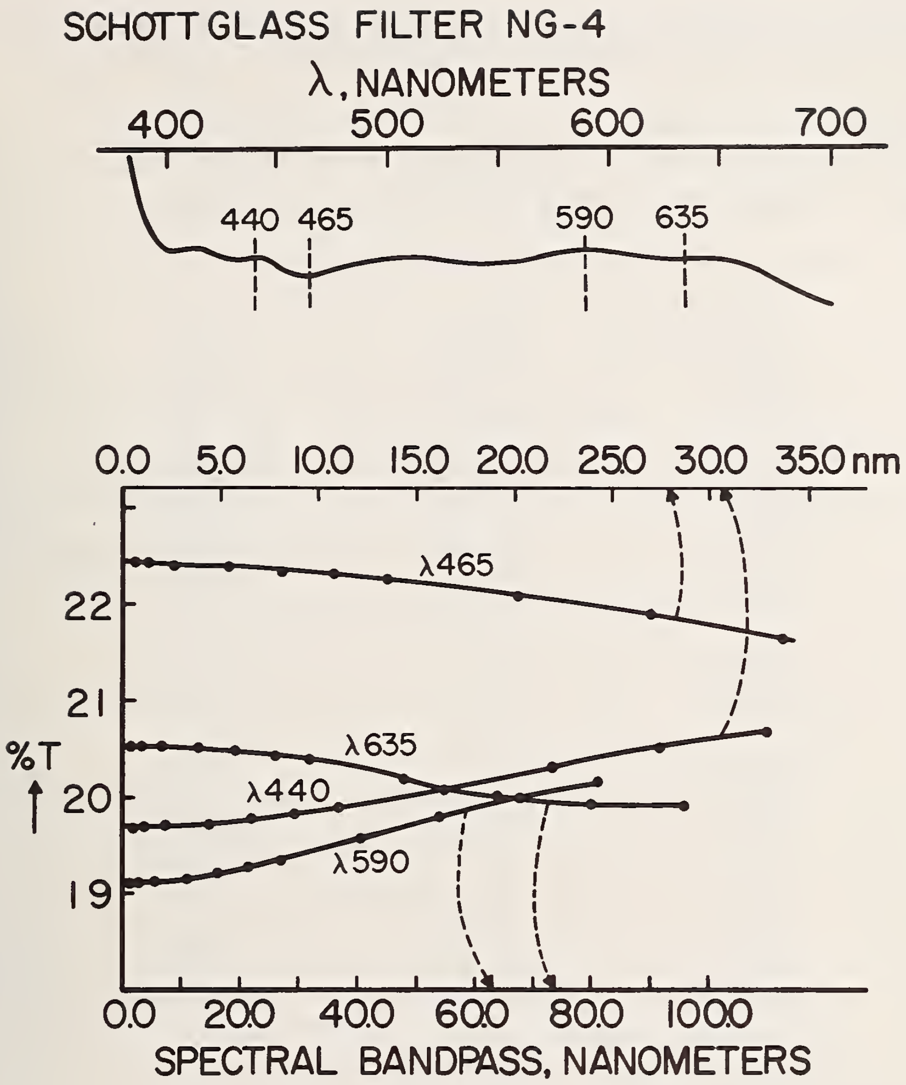

Figure 3. Optical transmittance versus spectral bandpass for a Schott NG-4 glass filter.

A series of measurements was then initiated to determine the proper procedure for initially cleaning the surface of the filters. Various wet and dry techniques were used and the following method was finally adopted. The glass filter was removed from its holder and placed on a lens paper. Each surface was then washed with redistilled ethyl alcohol followed by redistilled water. The surfaces were dried with lens paper. The filter was then replaced in its holder in the marked position (all manipulations were carried out using polyethylene gloves). The last traces of dust were removed with an artist's brush which was attached to a rubber hose connected to a vacuum source [4]. The cleaned filters were then placed successively in the spectrophotometer and transmittance readings were taken at the following wavelengths: 440, 465, 590 and 635 nm, using an effective spectral bandpass of 2.2, 2.7, 5.2 and 6.6 nm, respectively. Fifteen successive transmittance measurements were made at each wavelength and for each filter. The filters were then submitted to the cleaning procedure as described, followed by transmittance measurements and the entire operation was repeated four times. Since no blank was available, the measurements were performed against air. The results obtained on one set of filters are given in table 2.

From these results it can be seen that the percent transmittance can be measured on the glass filters with a standard deviation for a single determination not exceeding 0.041 percent. This value is not affected by successive cleanings of the filter surface using the washing procedure described. Measurements made on a similar set of three NG-4 filters have confirmed this observation; hence, the alcohol-water washing technique was adopted as a routine initial cleaning procedure for the glass filters. In subsequent use, if the surface appears, by visual inspection, to be free of contamination, the filter can be cleaned by merely breathing on its surface and wiping it with lens paper. The remaining traces of dust are eliminated with the brush-vacuum unit as described.

Several sets of Schott NG-4 filters were calibrated and one set was forwarded to members of the Standards Committee of the American Association of Clinical Chemists for preliminary evaluation.

Table 2. Effect of four consecutive washings on the transmittance measurements on three Schott NG-4 glasses.

Wash	Wavelength Å	% Transmittance[a]	Standard Deviation	% Standard Deviation
		Filter A		
First	4400	12.99	0.0053	0.041
	4650	15.04	.0042	.028
	5900	11.79	.0032	.027
	6350	12.80	.0032	.025
Second	4400	12.99	.0032	.024
	4650	15.04	.0052	.034
	5900	11.79	.0042	.036
	6350	12.80	.0042	.033
Third	4400	13.00	.0048	.037
	4650	15.04	.0042	.028
	5900	11.79	.0042	.036
	6350	12.80	.0042	.033
Fourth	4400	13.00	.0048	.037
	4650	15.04	.0042	.028
	5900	11.79	.0042	.036
	6350	12.81	.0042	.033
Average of Four Washings	4400	12.99	.0045	.035
	4650	15.04	.0044	.029
	5900	11.79	.0039	.034
	6350	12.80	.0039	.031
		Filter B		
First	4400	19.64	0.0042	0.021
	4650	22.37	.0053	.024
	5900	19.06	.0052	.027
	6350	20.46	.0053	.026
Second	4400	19.64	.0042	.021
	4650	22.37	.0048	.022
	5900	19.06	.0048	.025
	6350	20.47	.0048	.024
Third	4400	19.64	.0052	.026
	4650	22.37	.0042	.019
	5900	19.07	.0053	.028
	6350	20.47	.0048	.024
Fourth	4400	19.65	.0048	.025
	4650	22.38	.0053	.024
	5900	19.07	.0052	.027
	6350	20.47	.0053	.026

Table 2 continued

Average of Four Washings	4400	19.64	.0046	.023
	4650	22.37	.0049	.022
	5900	19.07	.0051	.027
	6350	20.47	.0050	.025

Filter C

First	4400	32.88	0.048	0.015
	4650	35.50	.0032	.009
	5900	31.23	.0042	.014
	6350	32.65	.0042	.013
Second	4400	32.87	.0052	.016
	4650	35.50	.0032	.009
	5900	31.22	.0048	.015
	6350	32.65	.0048	.018
Third	4400	32.87	.0052	.016
	4650	35.51	.0032	.009
	5900	31.22	.0042	.014
	6350	32.64	.0053	.016
Fourth	4400	32.87	.0048	.015
	4650	35.50	.0048	.014
	5900	31.22	.0048	.015
	6350	32.64	.0052	.016
Average of Four Washings	4400	32.87	.0050	.015
	4650	35.50	.0036	.010
	5900	31.22	.0045	.014
	6350	32.65	.0049	.016

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[a] Average of 15 individual measurements. Temperature = 25.2 °C

2. Evaporated Metals on Quartz

As seen from figures 1 and 2, the colored "neutral" glass filters have transmissions which still exhibit some dependence on wavelength.

The term "neutral" is used here in its loose meaning, only to indicate that the optical transmittance is less dependent on wavelength, when compared with other colored glass filters. Light filters exhibiting less transmission-wavelength dependence and nearer to optical neutrality can be produced by evaporating a thin semi-transparent layer of metal on a transparent substrate. Such filters have only a slight transmission-wavelength dependence, as illustrated in figure 1, where it can be seen that the maximum change in percent T is ± 4 over the spectral range of 2300 Å to 7500 Å. In this case, the evaporated metal was inconel and the substrate was non-fluorescent fused silica of optical quality. From the same figure it can be seen that the transmittance of these filters extends in the ultraviolet to 2300 Å which is an added advantage when compared with the colored glass filters. A limitation of the evaporated metal filters is that they reduce the intensity of a light beam by reflecting a part of it, rather than obsorbing. This can produce, in certain circumstances, undesirable stray light in the instrument and make the transmission measurements dependent on the geometry of the optical path (collimated, convergent, divergent). However, since these filters are closer to optical neutrality than the colored glass filters and since they can be used in the ultraviolet region as well, they were included in the studies to determine their suitability as SRMs.

a. Preparation of the Filters. A series of filters were prepared by the optical shop of the NBS according to the following specifications: a number of non-fluorescent optical quality silica plates, 10 mm x 25 mm and 1 mm thick, were cut and polished. Inconel metal was evaporated on the surface to produce nominal transmittances of 25 percent, 50 percent and 75 percent. The surface carrying the evaporated metal was coated with a layer of optical cement which was transparent to the visible and ultraviolet regions down to 2300 Å. A clear plate of the same material was used to cover and protect the evaporated metal layer.

The filter assembly was then marked at one corner to insure its positioning in the same place. The finished filter was then placed in a metal holder of conventional size (approximate o.d. = 13 x 13 x 57 mm) fitting the cell compartment of most spectrophotometers. The metal holder was also marked at one side to permit positioning of the filter in a reproducible manner.

In addition to the evaporated metal filters, a series of units were prepared using only the clear uncoated quartz plates and assembled with the same optical cement. These clear filter assemblies were then used as reference units in the blank compartment.

b. <u>Study of the Effect of Irradiation with Fluorescent Light</u>. Before submitting the evaporated metal filters to transmittance measurements, a study was made of the effect of radiations on the transmittance. A filter was exposed to an accelerated test in which radiations had the same spectral distribution as the fluorescent lighting of the laboratory, except that they were 1000 times more intense. The filter was exposed for an equivalent of 36,000 hours of continuous irradiation. This test was made on a radiation accelerometer made available by the Building Research Division of NBS. The percent transmittance was measured before and after the exposure and gave the following results:

	Transmittance, %T			
Wavelength, Å	2500	3800	5000	6500
Before exposure	44.48	51.35	48.90	47.41
After exposure	44.11	51.34	48.92	47.47

The relative standard deviation for a single determination of these measurements was 0.01 percent. As can be seen, the only measurable change in transmittance occurred at 2500 Å. This is probably due to the action of the intense radiation on the organic resin used in constructing the filter.

The change in percent T of about 0.84 percent at 2500 Å was considered as acceptable, since it is improbable that a filter would be exposed, in actual practice, to such intense radiation for such a long period. Several sets of evaporated metal filters were prepared and stored in plastic cases, as illustrated in figure 4.

c. Calibration of the Filter. Several sets of these filters were calibrated at five arbitrarily selected wavelengths, using the cleaning and measuring procedures outlined

Figure 4. Presentation of a set of provisional filters made from evaporated metal on quartz.

for Schott NG-4 colored glass neutral filters. Table 3 presents the results obtained for one of the filters having a nominal transmittance of 50 percent. Since similar results have been obtained for the other two filters with nominal transmittances of 25 percent and 75 percent, these are not given. As can be seen from this table, the reproducibility of transmittance measurements is good (percent standard deviation 0.009 to 0.024) and is comparable to those obtained for the colored glass filters at all wavelengths except 2500 Å. From the data in table 3 and from the additional measurements, it is evident that the transmittance of the evaporated metal filter at 2500 Å is a function of the cleanliness of the surface. The percent transmittance measured at 2500 Å varies from 43.99 to 44.14 after washing. The difference between these values is significant and well outside the expected average standard deviation determined at the various wavelengths. It is not possible, at present, to give a satisfactory explanation for the contributing cause of this phenomenon. Additional studies will be needed to determine the cause for this variation. It is recommended, however, that the

Table 3. Transmittance measurements on a filter of evaporated metal on quartz.

Wash	Wavelength and bandpass, Å		% Transmittance[a]	Standard deviation	% Standard deviation
First	2500	(3)	43.99	0.008	0.018
	3500	(10)	51.23	.006	.012
	4500	(24)	49.37	.006	.013
	5500	(44)	47.62	.006	.012
	6500	(70)	46.88	.004	.009
Second	2500	(3)	44.14	0.004	0.009
	3500	(10)	51.25	.012	.024
	4500	(24)	49.41	.012	.024
	5500	(44)	47.67	.009	.019
	6500	(70)	46.89	.004	.009

---

[a] Average of 10 measurements

surface of the evaporated metal on quartz filters be cleaned only by breathing lightly on its surface, wiping it with lens paper, and removing the dust particles with the vacuum brush.

From the several sets of evaporated metal on quartz filters calibrated on the conventional spectrophotometer, a set was examined by members of the Standards Committee of the American Association of Clinical Chemists.

It is well known that grid on quartz, polarizers and sectors will serve to check the photometric scale of spectrophotometers, and each has its advantages and limitations. Major attention was given, however, to the colored glass and evaporated metal filters. The activity will expand during the next year to include in our study the above materials.

C. Liquid Filters to Check the Photometric Scales of Spectrophotometers (R. W. Burke and E. R. Deardorff)

Solutions offer several unique advantages for calibrating the absorbance or transmission scales of spectrophotometers. Undoubtedly, their most important advantage is that they lend themselves to close duplication of conditions under which an unknown sample is measured. The sample cuvette, for example, with its potential sources of error, must be incorporated into the measurement procedure. Other advantages include: (1) absorbances or transmittances can be measured at any desired level simply by varying the concentration or cuvette path length, provided the Lambert-Beer law is obeyed, (2) provide a reference blank, (3) offer a direct means of calibrating flow systems, (4) conform to the shape of any cuvette and (5) present no inhomogeneity problems. Disadvantages which may be cited are: (1) solutions generally do not possess the permanence nor the high degree of optical neutrality that can be obtained with solid filters, (2) they require careful preparation and (3) they inherently exhibit much larger temperature coefficients than do solids.

1. Single Metal Solutions

The initial work on liquid filters concerned the reexamination of those solutions which have been investigated previously. These included potassium nitrate, acidic dichromate, basic chromate, cobalt sulfate and copper sulfate. The latter three solutions were studied at the National Bureau of Standards in the 1940's and absorbance-wavelength data for one concentration of each have been published [5]. The absorption spectra of these solutions are shown in figure 5. It is apparent that all absorbances are strongly dependent on exact wavelength settings, except in the regions near the peaks and valleys. Even in these less critical wavelength regions, the absorbance that one measures will be dependent on the size of the spectral bandpass employed. The latter can be calculated readily from the dispersion data supplied with the spectro-

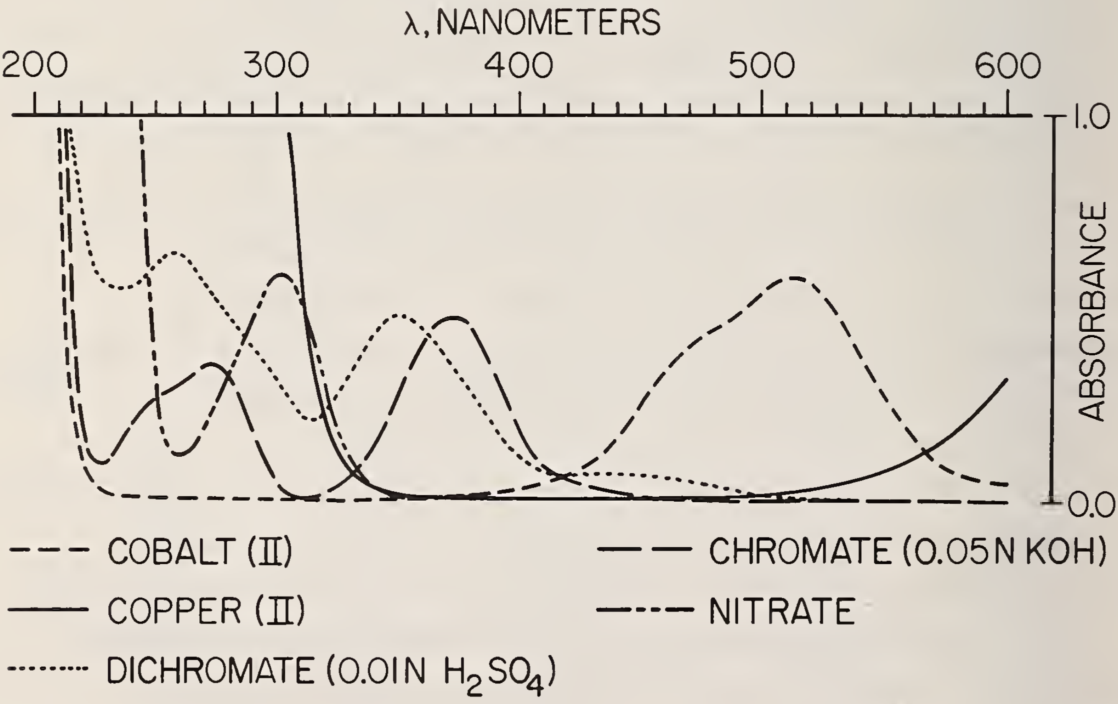

Figure 5. Absorption spectra of aqueous solutions of cobalt(II), copper(II), chromate, dichromate and nitrate.

photometer. For maximum signal-to-noise ratio one normally employs the largest bandpass commensurable with the degree of accuracy required. It has been shown that the spectral bandpass must not exceed 0.1 the natural bandwidth if 0.5 percent accuracy is desired [6]. For 0.1 percent accuracy this value reduces to 0.05.

The attractive characteristics of potassium nitrate, cobalt sulfate and copper sulfate are the excellent stability of both their salts and solutions, and their low molar absorptivities (weighing errors are thus minimized). Although potassium nitrate solutions may exhibit bacterial whiskers upon standing, these can be eliminated by previously boiling the water in which they are prepared. Acidic potassium dichromate and alkaline potassium chromate, likewise, display excellent stability when their solutions are free of oxidizable contaminants. Haupt [7], for example, observed no change above 260 nm in the spectral properties of alkaline chromate solutions when stored in glass containers for several years (an alkali-resistant glass is recommended, however). Both forms exhibit relatively high molar absorptivities and the concentrations normally used are of the order of 40-50 mg per liter. Apparently because of certain handling problems, the alkaline chromate solutions have not been used extensively. The tendency, instead, has always been to use the acidic form.

Several precautions must be observed in the use of acidic potassium dichromate as an absorbance standard. It has long been recognized that its apparent molar absorptivity varies with concentration. As early as 1934, Kortüm and von Halban [8] reported a variation of 23 percent (figure 6) in the molar absorptivity of dichromate over the concentration range 2×10^{-5} to 4×10^{-3}M when absorbance measurements were made at 366 nm (0.0025M sulfuric acid was used in these studies). This variation is attributed

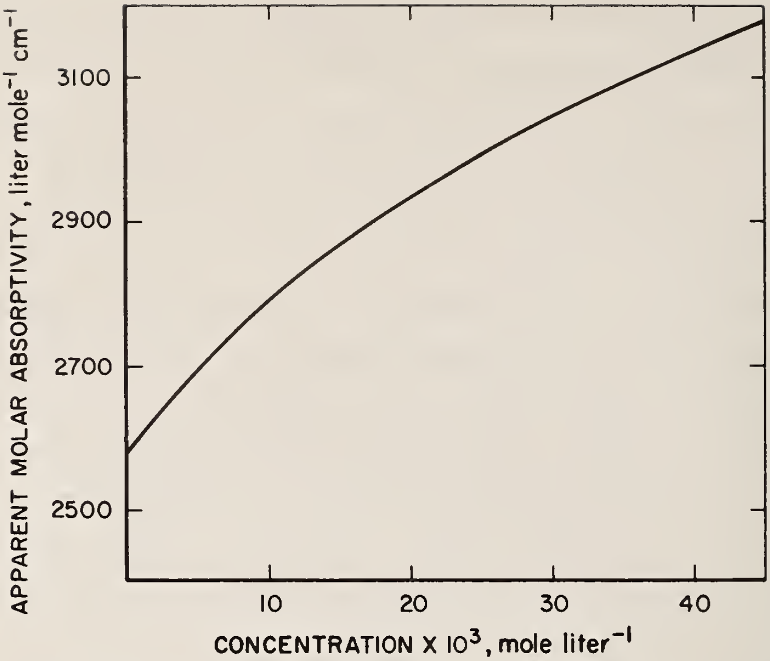

Figure 6. Apparent molar absorptivity of potassium dichromate as a function of concentration (from Kortüm and von Halban [8]).

primarily to the fact that the absorption spectra of dichromate ion and hydrogen chromate ion are significantly different (figure 7). Moreover, the fraction of each in an equilibrium solution is both concentration and acid dependent, as is evident from the following equilibria.

$$Cr_2O_7^{-2} + H_2O = 2HCrO_4^{-1} \qquad (1)$$

$$HCrO_4^{-1} = H^+ + CrO_4^{-2} \qquad (2)$$

Neuss and Rieman [9] have reported a value of 3.20×10^{-7} for the thermodynamic acidity constant (reaction 2) at 25°C of hydrogen chromate ion based on e.m.f. measurements. Their work also yielded a value of 2.32×10^{-2} for the equilibrium constant for reaction 1. More recent work by Davies and Prue [10] and Tong and King [11] suggests that this value is too low. Their values for 25°C are 3.03×10^{-2} and 2.82×10^{-2}, respectively. Furthermore, Tong and King

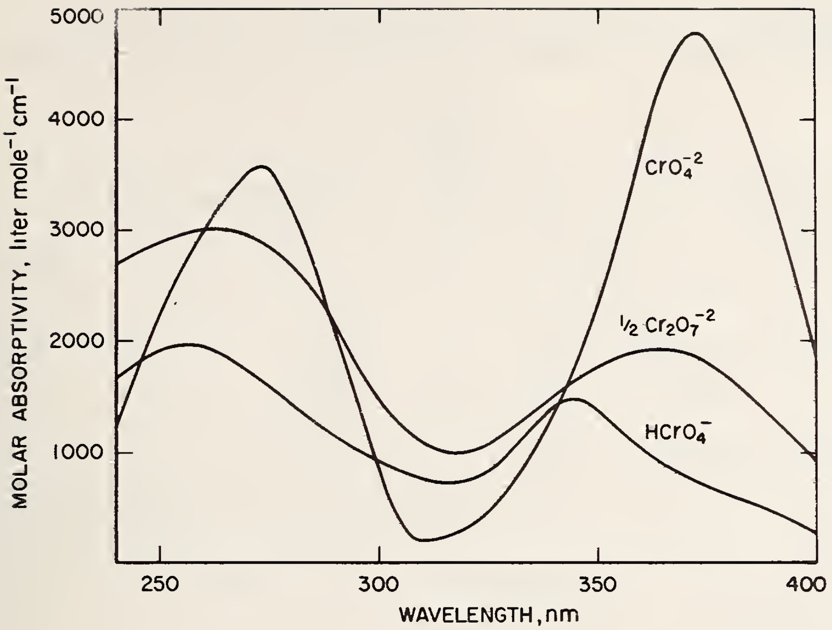

Figure 7. Absorption spectra of CrO_4^{-2}, $HCrO_4^-$ and $Cr_2O_7^{-2}$ (from Davies and Prue [10]).

noted in sulfuric acid media a dependence of K on the concentration of the acid. They suggest this effect may be due to the existence, even at very low acid concentrations, of mixed species containing sulfur and chromium. Thus, it is quite apparent that acidic dichromate should be used at a specified concentration, and the type and amount of acid should be clearly stated.

The molar absorptivities, calculated for aqueous solutions of potassium nitrate, acidic potassium chromate, alkaline potassium chromate, cobalt sulfate and cobalt perchlorate, are given in table 4. The value obtained for a nickel nitrate solution is also included. This solution and the two cobalt solutions were prepared from the metals ($\geq$99.99 purity) by dissolution in the designated acids. The final pH values of these solutions are also given in the table. All absorbance measurements were made at 25.0 ± 0.1°C in 10 mm cuvettes. The exact path lengths and parallelism of the cuvette faces were checked by mechani-

Table 4. Apparent molar absorptivities of selected aqueous solutions of inorganic salts at 25°C.

Salt	Concentration g/l	λ nm	Spectr. Band-pass nm	Molar[a] Absorpt.	Lit. Values	Ref.
KNO_3[b]	7.0-9.6	302	0.6	7.08	7.04-7.20 7.12 (Mean)	[12]
$K_2Cr_2O_7$[c]	0.050 (0.01 N H_2SO_4)	350	1.1	3150	3150	[13]
		257	0.4	4230	4270	[13]
K_2CrO_4[c]	0.040 (0.05 N KOH)	373	1.4	4800	4820	[13]
		273	0.5	3690	3570-3860 3690 (NBS)	[13] [7]
$Ni(NO_3)_3$[d]	6.8 as Ni (pH=2.7)	394	1.5	5.09	-	-
$CoSO_4$[d]	2.0 as Co (10 ml H_2SO_4)	513	2.0	4.87	-	-
$CoClO_4$	2.0 as Co (10 ml $HClO_4$)	513	2.0	4.82	-	

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[a] Tentative values
[b] J.F. Baker (special preparation for NBS)
[c] NBS-SRM 136a
[d] Prepared from metal

cal measurements and were accurate to ± 0.1 percent. The molar absorptivities given were obtained on a high precision spectrophotometer, the accuracy of which is not known at present. They should, however, provide a basis for comparison with values which have been obtained on other commercial instruments. As outlined later in this report, plans are underway at NBS for the design and construction of a high accuracy spectrophotometer.

2. Liquid Filters of Improved Optical Neutrality

Two approaches are being investigated to extend the wavelength and improve the optical neutrality of liquid filters. One utilizes composite solutions containing several materials, each of which exhibits one or more broad absorption bands. These materials are selected so that the absorption peak of one material complements the absorption minimum of a second component. The other approach employs water-soluble "black" dyes.

In 1946, Thomson [14] described the preparation of a composite inorganic grey solution consisting of chromic sulfate, cupric sulfate, cobaltous sulfate and potassium dichromate. We have reproduced this mixture and its absorption spectrum is shown in figure 8. The disadvantage of this solution is that it does not transmit below 300 nm. To develop a liquid filter that does exhibit a high degree of optical neutrality throughout the uv-visible spectrum, we have combined the chromium(III) and cobalt(II) components of the Thomson solution with a third component, p-nitrophenol, as the uv absorber in the 300 nm region. The absorption spectra of this solution (henceforth referred to as the NBS composite) and a Chance ON-10 glass are also shown in

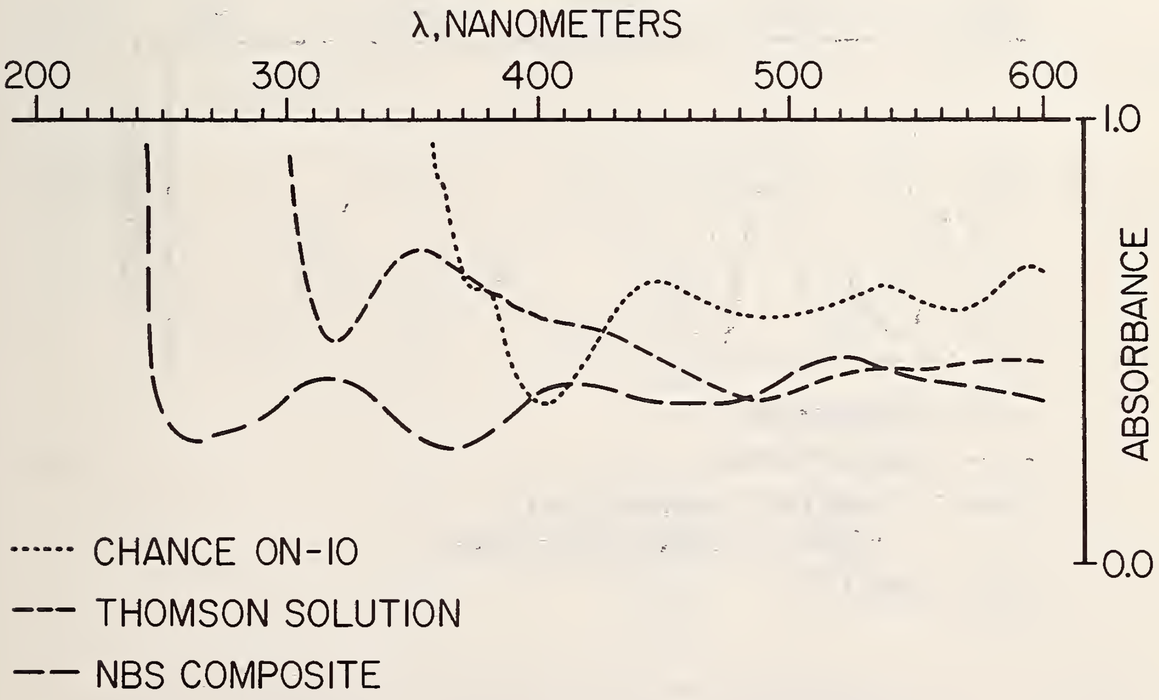


Figure 8. Absorption spectra of a Chance ON-10 glass, Thomson solution and an NBS composite solution.

figure 8. Over the range 250-600 nm, the NBS solution possesses a number of relatively broad maxima and minima, while the Chance glass filter does not transmit below 350 nm. The individual absorption spectra of the three components comprising the NBS solution are shown in figure 9. The absorption spectrum of a fourth solution, 2,7-dimethyl-3,6-diazacyclohepta-1,6-diene perchlorate is also shown in this figure. It was proposed originally for use as a uv filter by Schwarzenbach [15] and its solutions reportedly exhibit excellent stability. For our purposes, however, its absorption band is too narrow to compensate sufficiently for the broad chromium minimum that exists in the 300 nm region. p-Nitrophenol, however, does possess the necessary broad absorption band in this region. To date, the stability of its aqueous solutions meets present requirements.

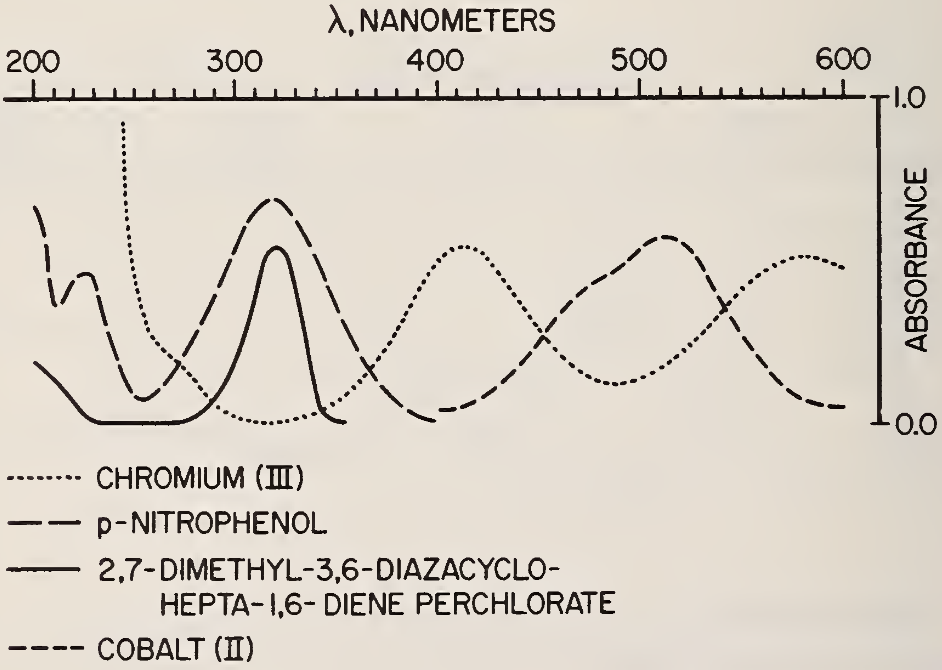


Figure 9. Absorption spectra of aqueous solutions of chromium(III), cobalt(II), 2,7-dimethyl-3-6-diazacyclohepta-1,6-diene perchlorate and p-nitrophenol.

Because of the presence of chromium(III) in both the Thomson and NBS solutions, an "aging" period is necessary to obtain spectral stability. About 6 to 8 weeks are required if this aging process is carried out in sulfate media at room temperature. Ways of reducing this equilibration time, however, are being investigated. Some success has been achieved in this respect, as will be noted in a subsequent section.

The absorbance of the Thomson and NBS solutions, both of which were prepared in sulfate media, have been measured periodically for three and one-half months following initial aging periods of about two months each. The values obtained at the beginning and end of this three- and one-half months period are given in table 5, with the interim absorbances falling between the extremes shown. The concentration of the Thomson solution measured was approximately a 3:8 dilution of Thomson's recommended formulation.

Table 5. Absorbances of a diluted Thomson solution and an NBS composite, initially[a], and after three and one half months.

Solution	λ, nm	Absorbance (1-cm cuvette)	
		Initially[a]	After 3.5 months
Thomson	640	0.280	0.282
	590	.320	.322
	540	.301	.303
	487	.242	.244
	410	.385	.386
	350	.525	.525
NBS Composite	525	0.562	0.564
	470	.397	.398
	415	.483	.484
	365	.252	.253
	320	.493	.493
	260	.256	.258

---

[a] Following an initial aging period of two months

The effect of temperature on the absorbance of the Thomson and NBS solutions has been measured at three wavelengths each (Thomson-590, 487 and 350 nm; NBS-525, 415 and 320 nm). The values obtained for the most temperature dependent wavelength of each are shown in figure 10. Results are included also for a Cibalan Black solution (see subsequent section). All values have been corrected for the relatively small contribution of density changes over the temperature range studied.

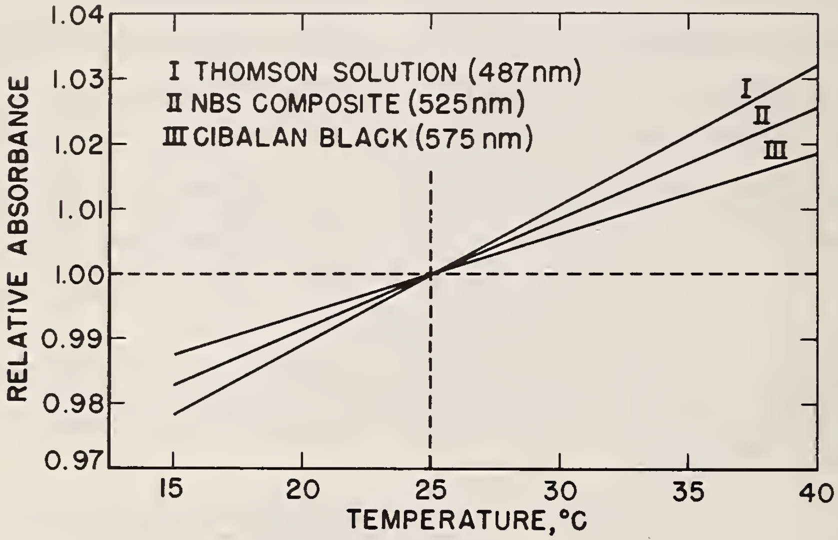


Figure 10. Effect of temperature on the absorbances of Thomson solution, an NBS composite solution and a Cibalan Black solution over the range 15-40°C.

3. Organic Solutions

In conjunction with the above program using various inorganic mixtures, an equally promising one using aqueous solutions of organic black dyes has also been initiated. The absorption spectra of several dyes which have been under investigation are shown in figure 11. All exhibit some preferential absorption at the ends of the wavelength region for which they are intended, i.e. 220-250 nm and

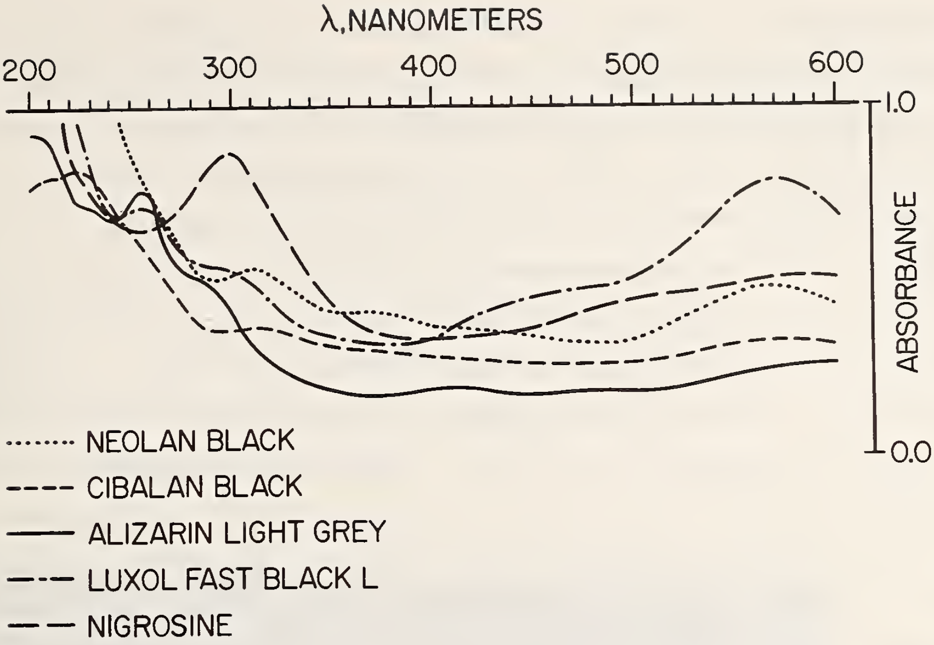


Figure 11. Absorption spectra of aqueous solutions of selected organic dyes.

550-600 nm. Because of this absorption in the red region of the spectrum, aqueous solutions of these dyes are not black but are various shades of blue. All of these dyes have relatively high molar absorptivities and typical concentrations are of the order of 40-60 milligrams per liter.

The Cibalan Black, Neolan Black and Alizarin Light Grey dyes have been the most extensively investigated to date. All are insensitive to variations in pH over the range 2-9, show no significant spectral differences after heating to boiling, and are relatively stable. Solutions constantly exposed to the fluorescent light of the laboratory showed absorbance variations of approximately 1 percent over a two months period.

The initial studies were carried out on samples of the commercial dyes, several of which contain as much as 40 percent impurities (some were added intentionally by the manufacturers to provide a fixed dye strength). With the assistance of the Organic Chemistry Section, present efforts are concerned with the purification of these dyes to such a level that, at least, the desired homogeneity can be obtained ($\leq$0.1 percent for 10-20 mg samples).

The effect of temperature on the absorbance of a recrystallized sample of Cibalan Black (figure 10) was measured at three wavelengths (315, 400 and 575 nm). Over the range of 15 - 40 °C, the largest temperature coefficient does not exceed 0.1 percent per °C. Commercial samples of Neolan Black and Alizarin Light Grey showed similar behavior.

The underlying goal of the current program involving liquid filters (both the inorganic composites and the black dyes) has been to develop potential absorbance standards that show a high degree of optical neutrality. Using the Chance ON-10 glass for comparison, the results shown in figure 12 indicate partial success.

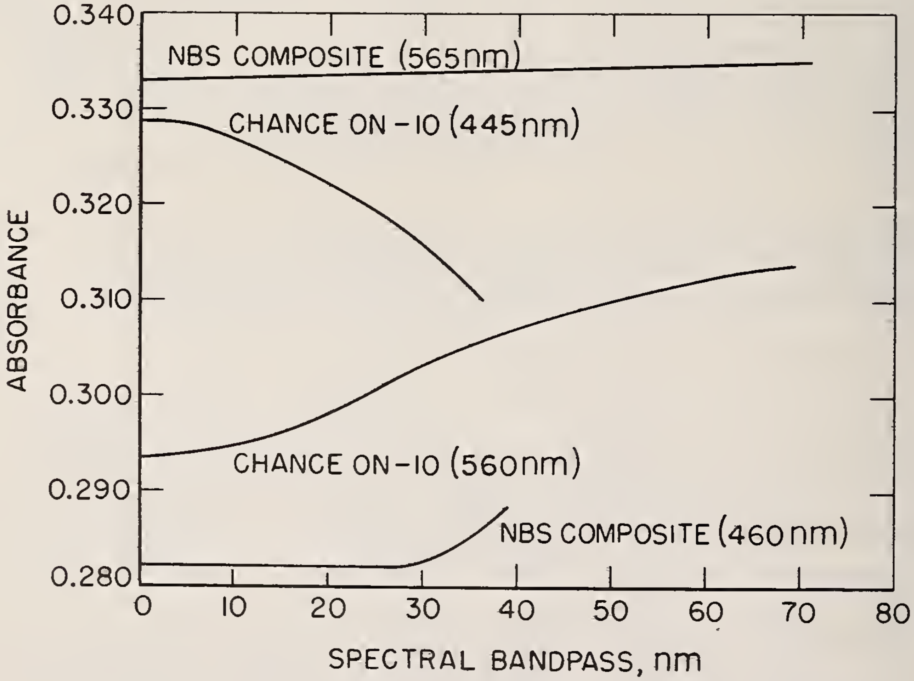


Figure 12. Comparison of the dependence of absorbance on the size of the spectral bandpass for Chance ON-10 glass and for an NBS composite.

The final step in the development of these, or similar liquid filters, will be to decide in what form they will be made available to the user. Depending on the outcome of the studies to establish rapidly equilibrium in chromium(III) systems, the inorganic composites could be provided either as solids or liquids. Because of the problem of accurately weighing milligram quantities of the black dyes, present plans call for preweighing and encapsulation of the samples which will be used to prepare "one-shot" solutions and then discarded. A possible package is shown in figure 13.

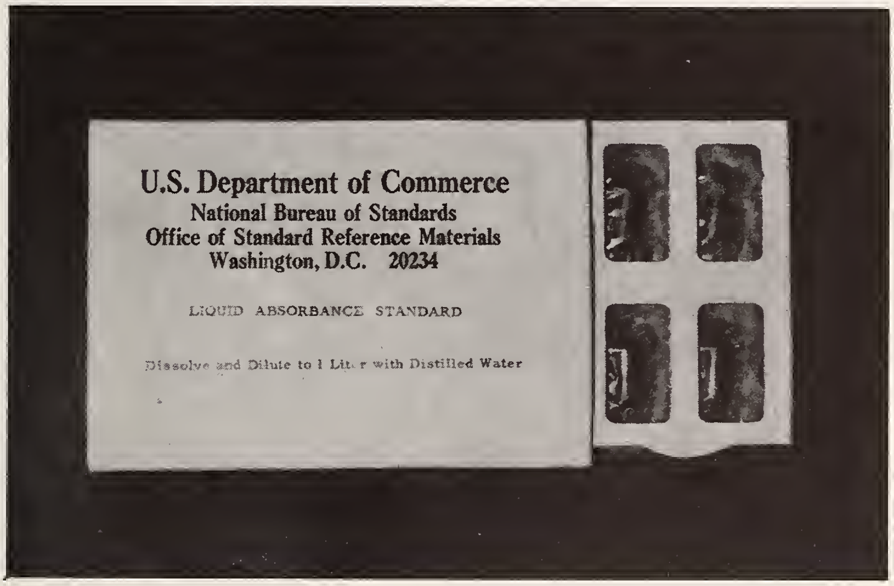
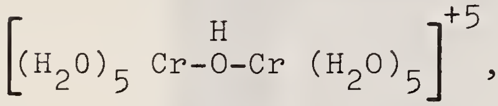


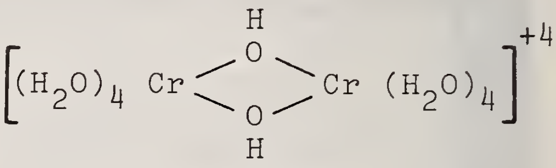
Figure 13. Suggested packaging for liquid absorbance standards.

4. Spectral Properties of Chromium(III) Solutions

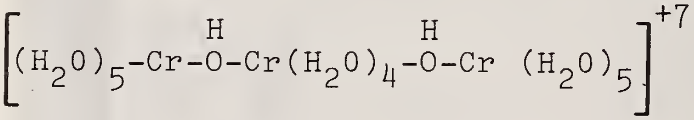
The two broad chromium(III) absorption bands in the visible spectrum, (maxima at approximately 410 and 580 nm) together with its good transmission in the ultraviolet, are promising characteristics which recommend the use of this ion in various mixtures which, in turn, can be used to prepare liquid filters of high optical neutrality. Unfortunately, solutions prepared from different chromic salts possess varying spectral properties and stabilities. It has been known for many years that aqueous solutions of chromic sulfate exist in both a violet and a green form. The green solutions of chromic sulfate are obtained either by heating or aging violet solutions. Also, it was recognized that the acidity of a boiled or aged solution of chromic sulfate was greater than that of a violet solution of the same chromium concentration. More recently, Hall and Eyring [16] used conductometric titrations to study the constitution of chromic salts in aqueous solutions. They present strong evidence that, during the aging process, the hexaaquo chromium(III) ions polymerize to yield species such as those shown below.



$$\left[(H_2O)_5\ Cr-\overset{H}{O}-Cr\ (H_2O)_5\right]^{+5},$$



and



$$\left[(H_2O)_5-Cr-\overset{H}{O}-Cr(H_2O)_4-\overset{H}{O}-Cr\ (H_2O)_5\right]^{+7}$$

The observed increase in acidity is explained by the formation of hydroxyl bridges. Hall and Eyring also showed that, after refluxing a chromic nitrate solution for 66 hours, the titration results were no longer affected by subsequent heating.

Because of the success of Hall and Eyring's experiments, we decided to investigate the effect of heating on the spectral properties of chromium(III) solutions. The bromide, chloride, perchlorate and sulfate salts were used. Absorption spectra were obtained periodically on portions of each as a function of storage time at room temperature. The remaining portions were refluxed for various periods of time and their absorption spectra were recorded. One effect common to both of these experiments was that the acidity of all solutions increased upon standing, the largest changes occurring in the refluxed samples. The absorption spectra of these chromic salt solutions are shown in figures 14, 15, 16 and 17 together with the conditions under which they were obtained. Also shown in figure 17 is the absorption spectrum of a chromium(III) solution prepared by dissolving the metal in sulfuric acid and aging at room temperature for two months. The transmission of this solution extends

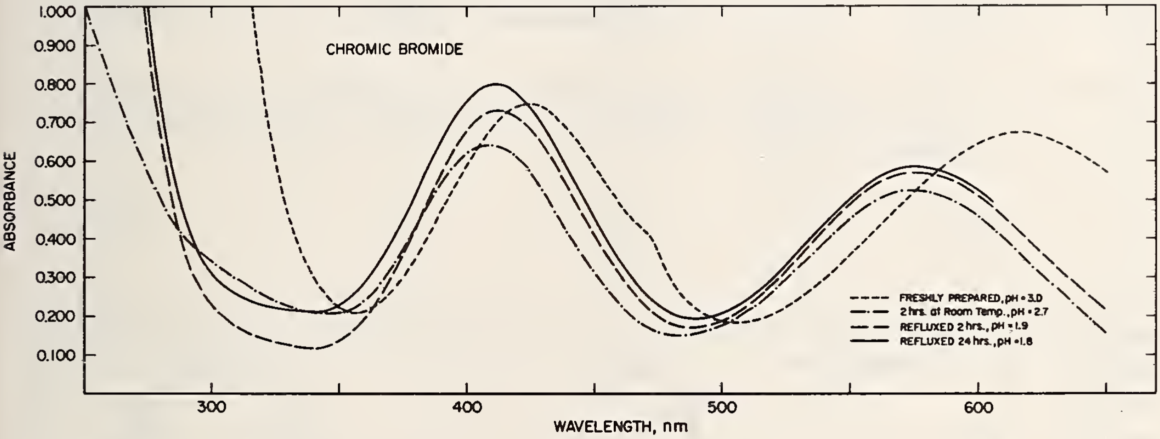


Figure 14. The absorbance spectra of an aqueous chromic bromide solution, initially, and after refluxing.

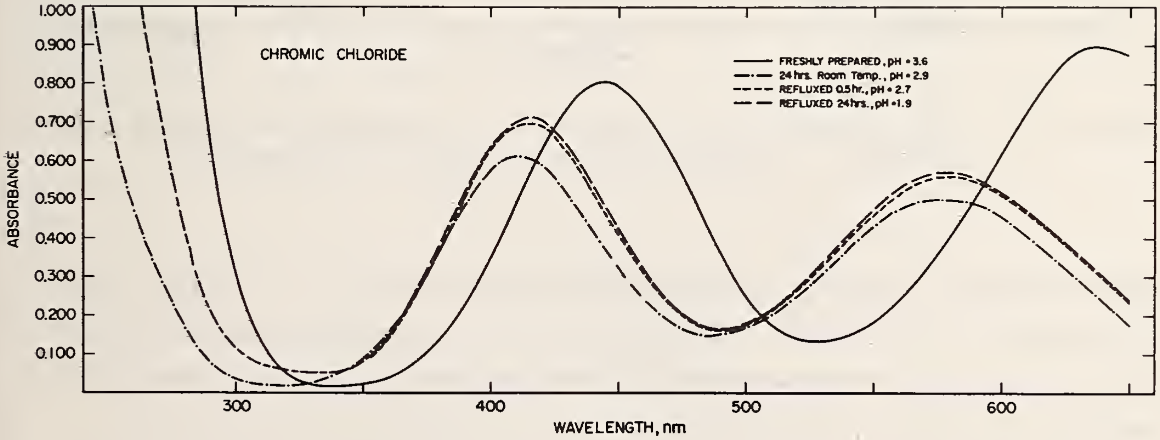


Figure 15. The absorbance spectra of an aqueous chromic chloride solution, initially, and after refluxing.

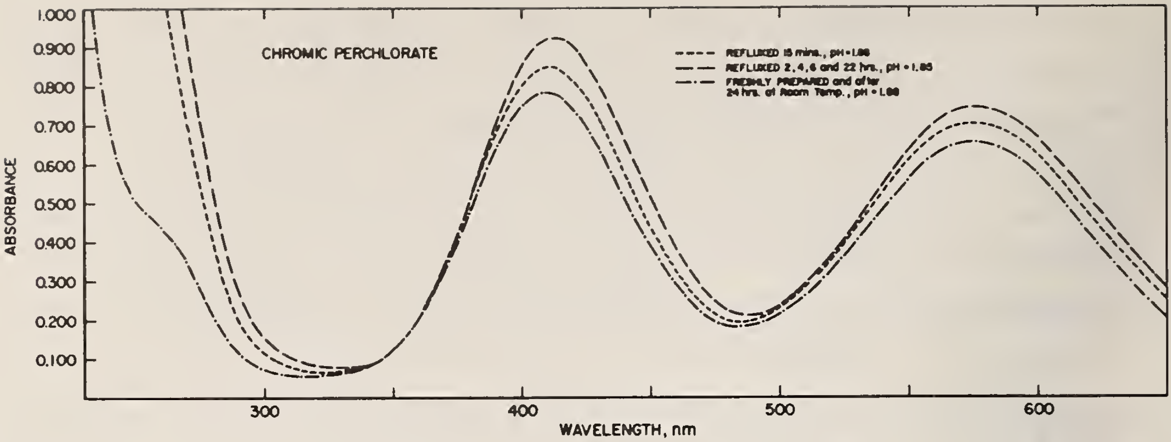


Figure 16. The absorbance spectra of an aqueous chromic perchlorate solution, initially, and after refluxing.

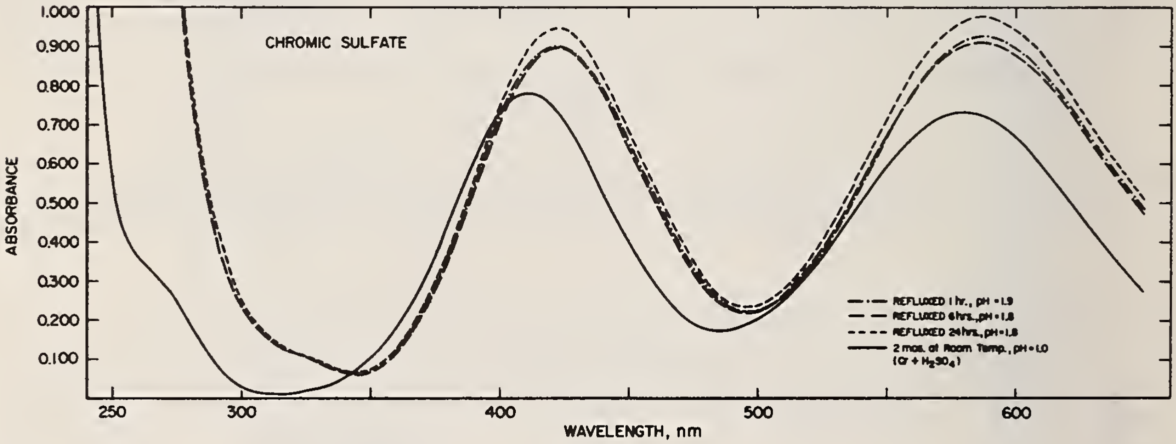


Figure 17. The absorbance spectra of aqueous solutions of chromic sulfate after various treatments.

significantly farther into the ultraviolet than do any other of the salt solutions. There is a similarity, however, in the ultraviolet between this aged solution and the freshly prepared chromic perchlorate. The significance, if any, that can be attributed to the difference in pH of these two solutions (sulfate pH 1; perchlorate pH 2.7) needs to be determined.

Table 6. Intercomparison of results obtained on chromium(III) solutions stored at room temperature.

Solution	Storage time, h	A_s max, nm λ_1	λ_2	A_s ratio λ_1/λ_2	pH
Chromic Perchlorate	0	409	573	1.19	2.72
	24	409	576	1.19	2.72
	48	409	576	1.20	2.70
Chromic Chloride	0	445	635	0.92	3.40
	24	410	576	1.23	2.88
	48	410	576	1.20	2.70
Chromic Bromide	0	428	624	1.13	2.70
	24	408	576	1.23	2.60
	48	408	576	1.29	2.42
Chromic Sulfate	0	421	587	0.98	2.34
	24	421	587	0.98	2.30
	48	421	587	0.98	2.25
Chromium in H_2SO_4	2 mos.	413	580	1.06	1.01

To facilitate the intercomparison of the spectral data obtained, the results have been summarized in tables 6 and 7 for the unheated and refluxed solutions, respectively. It is interesting to note that the final pH of the four refluxed chromic salt solutions are quite similar. However, in the case of the unheated solutions, the pH of the sulfate is somewhat lower than the other three. The fact that the ratio of peak absorbances in chromic perchlorate and chromic chloride solutions appear to be identical after refluxing for only one hour strongly suggests that spectral stability may be readily achieved in these two systems. The bromide and sulfate systems, on the other hand, appear too complicated to warrant further study at this time.

Table 7. Intercomparison of results obtained on chromium(III) solutions after refluxing.

		A_s max, nm		A_s ratio	
Solution	Time, h	λ_1	λ_2	λ_1/λ_2	pH
Chromic Perchlorate	0.25	411	576	1.21	1.88
	1.0	413	578	1.25	1.85
	6.0	413	578	1.25	1.85
	24.0	413	578	1.25	1.85
Chromic Chloride	0.5	413	579	1.24	2.69
	1.0	413	579	1.25	1.95
	6.0	413	579	1.25	1.90
	24.0	413	579	1.25	1.90
Chromic Bromide	1.0	413	578	1.25	2.00
	2.0	413	578	1.29	1.88
	6.0	413	580	1.35	1.82
	24.0	413	580	1.37	1.80
Chromic Sulfate	0.5	423	587	0.97	2.20
	1.0	423	587	0.97	1.90
	6.0	423	587	0.99	1.88
	24.0	423	587	0.97	1.88
	50.0	422	586	0.98	1.85
	76.0	422	586	0.98	1.88

D. Instrumentation (R. Mavrodineanu)

1. Proposed Design and Construction of High Accuracy Spectrophotometer

The transmittance data discussed in Part B were obtained with a commercial instrument in which the transmittance scale from 0 to 100 is divided into 1000 divisions. The stability is such that one division (or less) or one part per thousand can be read.

The repeatability or precision of the measurements from tables 2 and 3 and figures 1, 2 and 3 are thus determined to one part per thousand. This is confirmed by the values for standard deviations given in those tables.

The accuracy of the transmittance data could not be determined, however, since no independent means was available to determine this number; thus, the transmittance values discussed here have only a relative meaning.

The accuracy of the transmittance measurements is a parameter of fundamental significance in characterizing a chemical species through its molar absorptivity, as well as allowing the interchange and communication of data between laboratories. Hence, it was decided to initiate the design of a spectrophotometer capable of providing data of known accuracy and precision, and to construct such an instrument. The first phase of this program has been completed and the design of the spectrophotometer was established. It is hoped that the construction and testing of an instrument can be completed during Fiscal Year 1971.

2. Cell Light Path

An important factor in spectrophotometric measurements is the length of the light path. This obviously depends on the inside dimensions of the glass or quartz cell used. Therefore, particular attention was given to this factor and various procedures were developed to determine the width of the absorption cells of various shapes and sizes. This work was done in cooperation with Mr. J. Matwey of the Analytical Chemistry Division.

For rectangular cells, a feeler gauge using a single and double probe with a dial reading was used. The path length of circular cells was determined by microscopic techniques using translation measurements between the inside faces of the cell.

Since the rectangular absorption cells with a 10 mm path length are used in the vast majority of routine measurements, a simple and practical apparatus was designed and is now being assembled to measure this parameter. It consists of a twin probe unit provided with a dial reading to 0.01 mm. The dial is set to indicate 10.00 mm and any deviation from this value is read as plus or minus values within 0.01 mm, from 9.90 mm to 10.10 mm. The probe is calibrated for accuracy against an NBS reference block to ± 0.001 mm tolerance. Hence the measurements of the path length are accurate to at least 0.1 percent.

The twin probe is placed on a sliding plate which can translate in and out of the cell. The cell itself is located on the same plate in front of the probe between two spring-loaded metal blocks. Due to its simplicity and ease of manipulation, such a measuring tool could be a useful instrument in laboratories where spectrophotometric determinations are carried out.

E. Spectrophotometric Determination of Trace Amounts of Gold, Iron, Manganese and Nickel in Two Glass Standard Reference Materials (R. W. Burke and E. R. Deardorff)

Two sets of glass Standard Reference Materials were analyzed spectrophotometrically for gold, iron, manganese and nickel. The approximate composition of the base glass was 72 percent SiO_2, 12 percent CaO, 14 percent Na_2O and 2 percent Al_2O_3. In addition to this base composition, equal amounts of 61 other elements were added to each melt in order to obtain nominal doping levels of 500 and 50 ppm.

The glasses were in the form of wafers about 1 cm in diameter. Two thicknesses were provided: 1 mm sections, weighing 200-300 mg and 3 mm sections weighing approximately 1 g. Following weighing and transferring to 30-ml Teflon beakers, the samples were cleaned by the recommended procedure (the wafers were rinsed successively with 95 percent ethanol, distilled water, dilute nitric acid (1+9) and distilled water). Ten ml of hydrofluoric acid (1+1) were added, the beakers were covered with Teflon lids and placed on a hot plate at 50-60°C. To hasten dissolution, the wafers were tapped occasionally with a platinum rod to remove the insoluble fluoride coatings (calcium and rare earth fluorides) which formed. The resulting solutions, frequently containing small amounts of insoluble salts, were then evaporated to dryness. For the spectrophotometric determination of gold and nickel, 4 ml of aqua regia were added and the solutions were again evaporated to near dryness. Two ml of hydrochloric acid (1+1) were added and again evaporated. The residues were dissolved in 1N hydrochloric acid, transferred to 100 ml volumetric flasks and diluted to volume with 1N HCl. Any insoluble salts which were present dissolved upon standing (several days were necessary in some instances).

Prior to the spectrophotometric determination of iron, one of the chief concerns was the magnitude and reproducibility of the iron blank. Two precautions were taken. All dissolutions were performed in a Teflon-lined hood and only Merck "Suprapur" reagents, or the equivalent, were used. Following dissolution and evaporation of excess hydrofluoric acid, 2 ml of concentrated perchloric acid were added and the solutions were heated to strong fumes (approximately 1 ml of the perchloric acid was evaporated). The samples were transferred to 100 ml volumetric flasks with distilled water and diluted to volume. Manganese was also determined in these solutions. All standard solutions (gold, thallium, iron, manganese and nickel) were prepared from the high purity metals.

1. Spectrophotometric Determination of Gold

Gold was determined spectrophotometrically in the 500 and 50 ppm glasses following extraction of the [Brilliant Green]$^+$ $AuCl_4^-$ complex into benzene [17]. Since thallium(III) quantitatively accompanies gold(III) in the extraction procedure, corrections were applied based on the isotope dilution-mass spectrometric values reported previously for thallium by the Analytical Mass Spectrometry Section (62.0 and 15.8 ppm, respectively). The standard solutions of gold(III) and thallium(III) were prepared by dissolving the metals in aqua regia and then removing the excess nitric acid by repeated evaporations with hydrochloric acid.

A more desirable approach for the spectrophotometric determination of gold with Brilliant Green is, undoubtedly, to eliminate the thallium(III) interference. At least two possibilities seem worthy of further study. One is the selective reduction of thallium(III) to thallium(I) without the simultaneous reduction of gold(III); the other is the pre-extraction of thallium(III) following an exchange reaction with one of the metal chelates of dibenzyldithiocarbamic acid, possibly the zinc complex.

The spectrophotometric results obtained for gold in the 500 and 50 ppm glasses are given in tables 8 and 9, respectively.

2. Spectrophotometric Determination of Iron

The bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) procedure of Diehl and Smith [18] was employed. The iron(III) in a 10 ml solution (pH∿1) was reduced to iron(II) with hydroxylamine hydrochloride (2 ml of a 10 percent aqueous solution) and the pH adjusted to approximately 4.5 by the addition of 4 ml of 10 percent aqueous sodium acetate. Four ml of 0.001M bathophenanthroline (reagent initially dissolved in absolute ethanol and then diluted two-fold with distilled water) were added and the red iron(II)-bathophenanthroline complex was extracted into 6 ml of n-hexyl alcohol, using 30 ml separatory funnels equipped with all Teflon fittings. Approximately 15 minutes were allowed for the two phases to separate completely and then the bottom (aqueous) layer was drawn off and discarded. The n-hexyl alcohol phase was transferred to 10 ml volumetric flasks, the separatory funnels (including stoppers) were rinsed with small portions of ethanol and the combined extracts and washings were diluted to volume with ethanol. Absorbance measurements were made at 535 nm, using 1.00 cm cuvettes.

An important advantage of the good extractability of the Fe(II)-bathophenanthroline complex is that it permits the removal of trace amounts of iron from the reagents used in the complexation reaction. The aqueous solutions of hydroxylamine hydrochloride and sodium acetate were freed of iron in this manner. The latter solution, in particular, contained relatively large amounts of iron and at least a dozen extractions were necessary before the n-hexyl alcohol phase became colorless. Following this treatment, the combined reagent blank was 0.007 absorbance units, i.e. 0.18 mg of iron. The total blank (reagent + environmental) obtained following dissolution of the glass samples in the laminar flow, Teflon-lined hood was not distinguishable from the combined reagent blank, thus establishing that no detectable air-borne contamination with iron occurred.

The spectrophotometric results obtained for iron in the two glass Standard Reference Materials are given in tables 8 and 9.

Table 8. Spectrophotometric determination of gold, iron, manganese and nickel in 500 ppm glass.

Sample No.	Gold ppm[a]	Iron ppm[c]	Manganese ppm[d]	Nickel ppm[a]
2	37.0	460	481	445
13	33.7	458	485	451
18	33.9	453	485	457
48	35.3	459	487	450
56	34.3	463	492	446
66	34.3	468	487	448
78	54.9[b]	468	482	457
	41.8[b]			
	45.2[b]			
106	39.0	457	481	456
Average	35.4[e]	460.8	485.0	451.3
Std. Dev.	2.0	5.3	3.7	4.9
Rel. Std. Dev.	5.5%	1.1%	0.8%	1.1%

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[a] Sample weights 0.2-0.4g; 10/100 aliquots taken for analysis.

[b] Successive samples.

[c] Sample weights 1.0-1.5g; 2/100 aliquots taken for analysis.

[d] Sample weights 1.0-1.5g; 10/100 aliquots taken for analysis.

[e] Results on sample 78 not included.

Table 9. Spectrophotometric determination of gold, iron, manganese and nickel in 50 ppm glass.

Sample No.	Gold ppm[a]	Iron ppm[b]	Manganese ppm[c]	Nickel ppm[a]
2	9.4	49.0	40.0	37.7
37	9.7	51.2	39.7	37.8
44	10.8	51.8	39.7	37.7
68	10.0	52.8	39.5	37.5
75	9.0	52.6	39.3	37.3
114	9.7	52.8	39.1	37.8
Average	9.8	51.7	39.6	37.6
Std. Dev.	0.6	1.5	0.3	0.2
Rel. Std. Dev.	6.2%	2.8%	0.8%	0.5%

---

[a] Sample weights 0.8-1.3g; 10/100 aliquots taken for analysis.
[b] Sample weights 0.9-1.3g; 10/100 aliquots taken for analysis.
[c] Sample weights 0.9-1.3g; 25/100 aliquots taken for analysis.

3. Spectrophotometric Determination of Manganese

Manganese was determined spectrophotometrically following oxidation of manganese(II) to permanganate with ammonium persulfate-potassium periodate. The detailed procedure is as follows: A suitable aliquot of the manganese solution is transferred to a 125 ml Erlenmeyer flask followed by the addition of 1.5 ml concentrated sulfuric acid, 0.5 ml concentrated phosphoric acid and 0.2 ml concentrated nitric acid. Several crystals of silver nitrate and 0.5 g of ammonium persulfate are added and the resulting solution is diluted to approximately 40 ml with distilled water. The solution is boiled over an open flame for 5 minutes, removed from the heat, 0.3 g potassium periodate added, again boiled over the flame for an additional 2 minutes and then warmed for 15 minutes on a steam bath. The solution is cooled, transferred to a 50 ml volumetric flask and diluted to volume with 0.1N nitric acid (the latter was pre-boiled with ammonium persulfate-potassium periodate). A blank was treated in a similar manner. The net absorbance at 545 nm was then determined using 10.00 cm cuvettes (the chromium present did not interfere and the usual sodium azide reduction of permanganate was not employed).

The spectrophotometric results obtained for manganese in the 500 and 50 ppm glasses are given in tables 8 and 9, respectively.

4. Spectrophotometric Determination of Nickel

Nickel was determined spectrophotometrically by a modified dimethylglyoxime procedure which has been described in detail previously [19]. The complexation reaction is carried out at pH 13, using ammonium citrate as a masking agent. No separations were necessary in the analysis of these two glass SRM's. Absorbance measurements were made at 465 nm in 5.00 cm cuvettes. The results are given in tables 8 and 9.

2. FLUORESCENCE

A. Introduction (R. A. Velapoldi)

Spectrofluorimetry is at the same place that spectrophotometry was twenty-five years ago. It is quite apparent from the growth in publications in the past few years that it is rapidly approaching spectrophotometry in importance as an analytical tool. Research and use of spectrofluorimetry is carried out in such diverse fields of interest as clinical chemistry and testing, environmental chemistry (defense and pollution), forensic science and analytical chemistry.

Spectrofluorimetry generally is more sensitive than most spectrophotometric techniques since it depends on amplification of a signal rather than amplification of a small difference or ratio between two signals. Thus, metals such as magnesium or aluminum have been determined at the nanogram level or less. Spectrofluorimetry also has greater specificity. Analyses depend not only on the emission wavelengths, but also on the exciting wavelengths.

Many fluorescence spectra reported are uncorrected and incorporate errors produced by instrumental artifacts such as photomultiplier inefficiencies, light source instabilities and monochromator aberrations. It is imperative that fluorescence standards and corrected spectra be obtained [20].

Prerequisites for fluorescence standards include stability, solubility in aqueous and organic systems, ease of purification, a relatively broad fluorescence spectrum, excitation and fluorescence in the same general wavelength regions as the compound under investigation and a constant quantum efficiency, Q_f, as a function of exciting wavelength [21].

Quinine sulfate is one of the most widely used and accepted standards, although recently some concern has been raised on the variations of Q_f with exciting wavelength and its instability in solution at low concentration. In addition, reported Q_f values of 0.4 to 0.6 exemplify the need for further study on this system.

Fluorescence band spectra are somewhat narrow (100 nm at half band width); thus development of a series of fluorescent standards which cover the 250-750 nm region is needed.

B. <u>Program</u>

In view of the requirements in the field of fluorescence, an outline of a program was proposed and is presented in table 10 which has as its main objectives the development of SRMs, the compilation and publication of corrected fluorescence spectra with use of computer technology, and the investigation of new analytical techniques. As can be seen, development of solution and solid (glasses) fluorescent SRMs is planned.

The corrected spectra of two possible types of SRMs are given in figures 18-21. Figure 18 shows spectra of

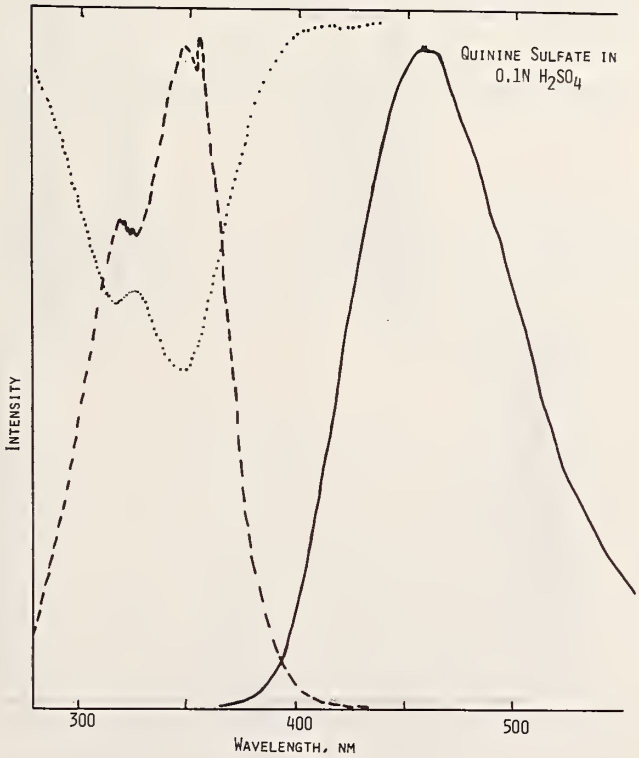
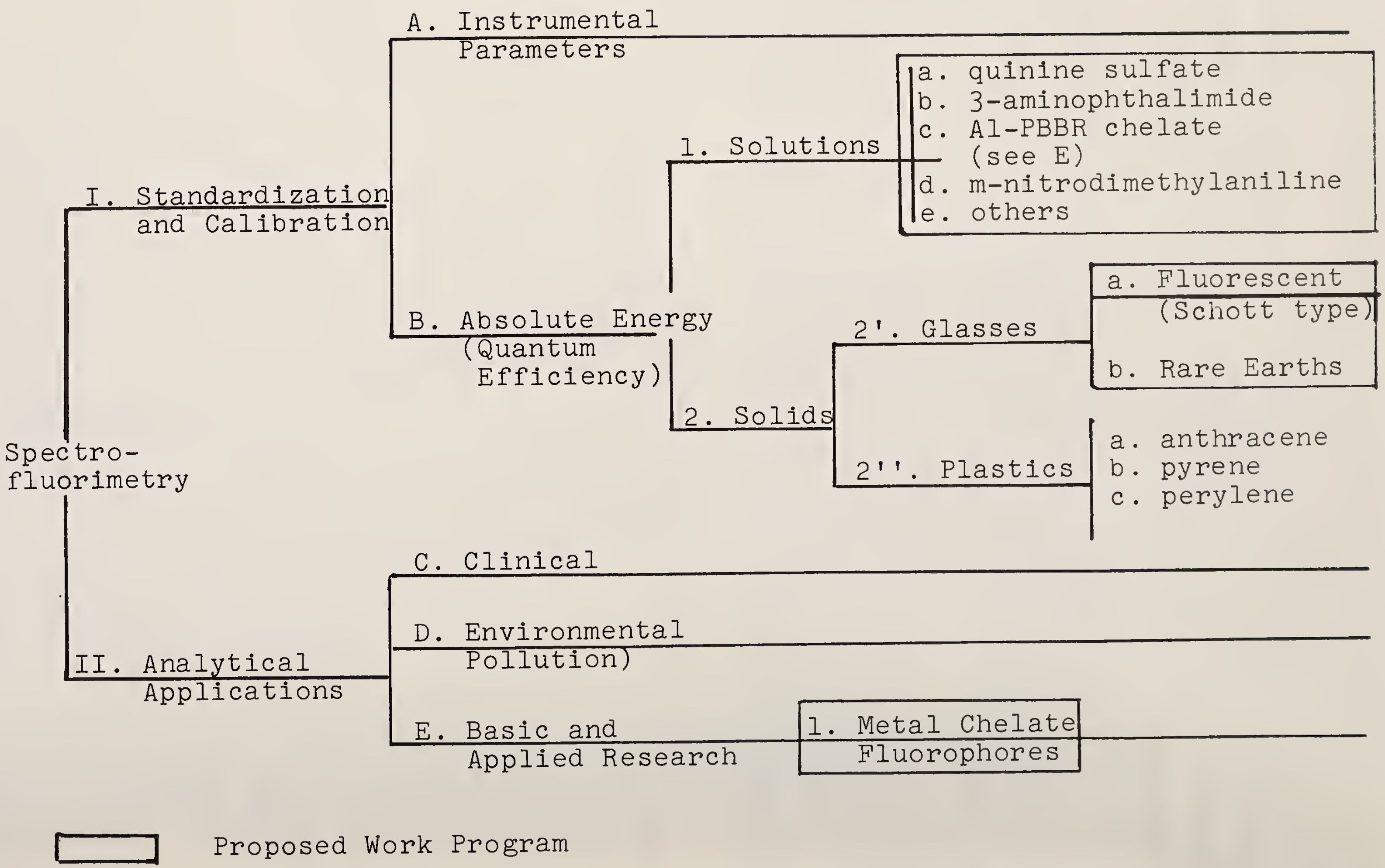


Figure 18. Corrected emission ———, excitation ----- and absorption ..... spectra of quinine sulfate in 0.1N sulfuric acid.

Table 10. Outline of the proposed program.



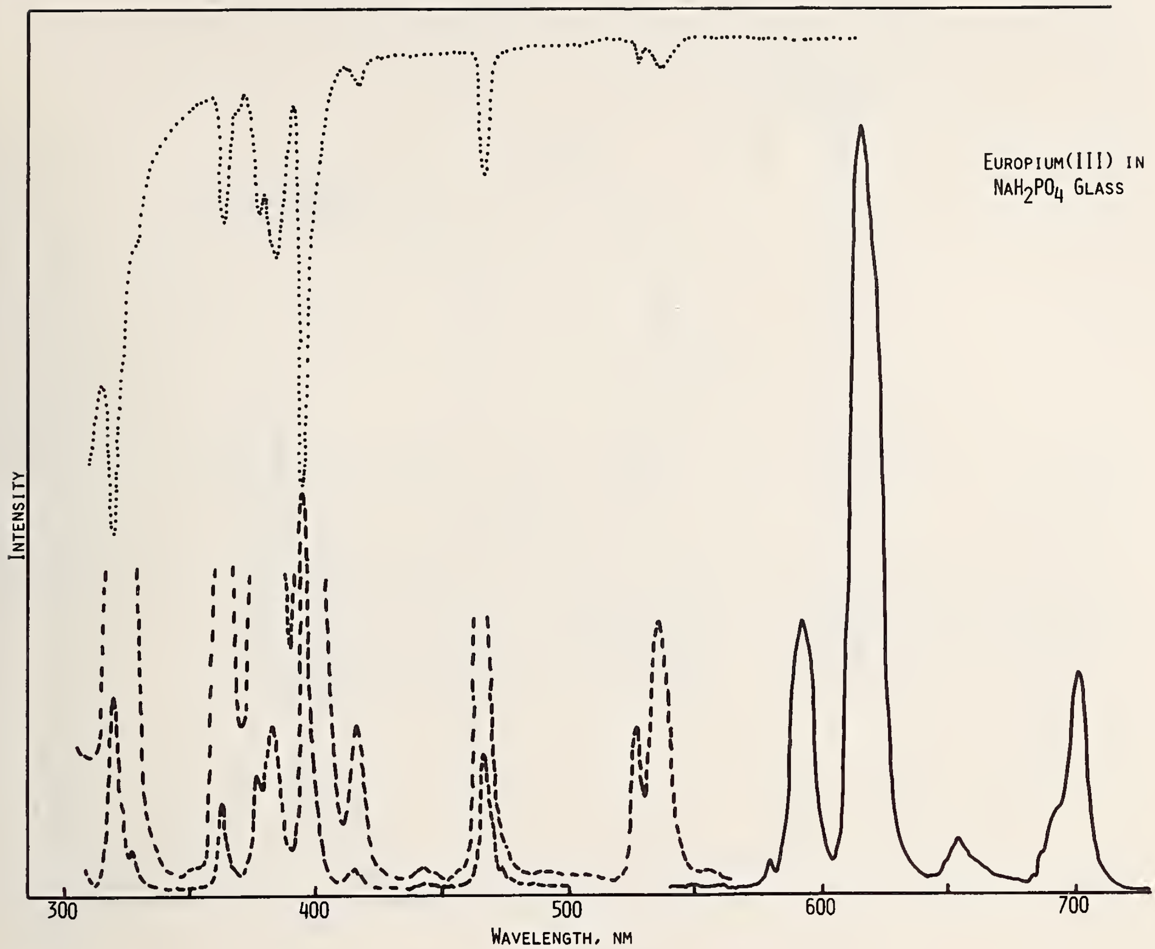


Figure 19. Corrected emission ———, excitation ----- and absorption ..... spectra of europium(III) in a NaH_2PO_4 glass prepared at Hebrew University, Israel.

quinine sulfate in 0.1N H_2SO_4. The absorption and excitation spectra are identical, except for the sharp excitation peak at 355 nm. Further work will be undertaken to determine whether this peak is an instrumental artifact or whether it is the hidden electronic transition [22] responsible for the change of Q_f with exciting wavelength.

The spectra presented in figures 19 and 20 are typically produced by lanthanides and are exemplified by europium(III) in silicate and phosphate glasses. The Eu(III) in phosphate glass was obtained from Dr. Renata Reisfeld at the Hebrew

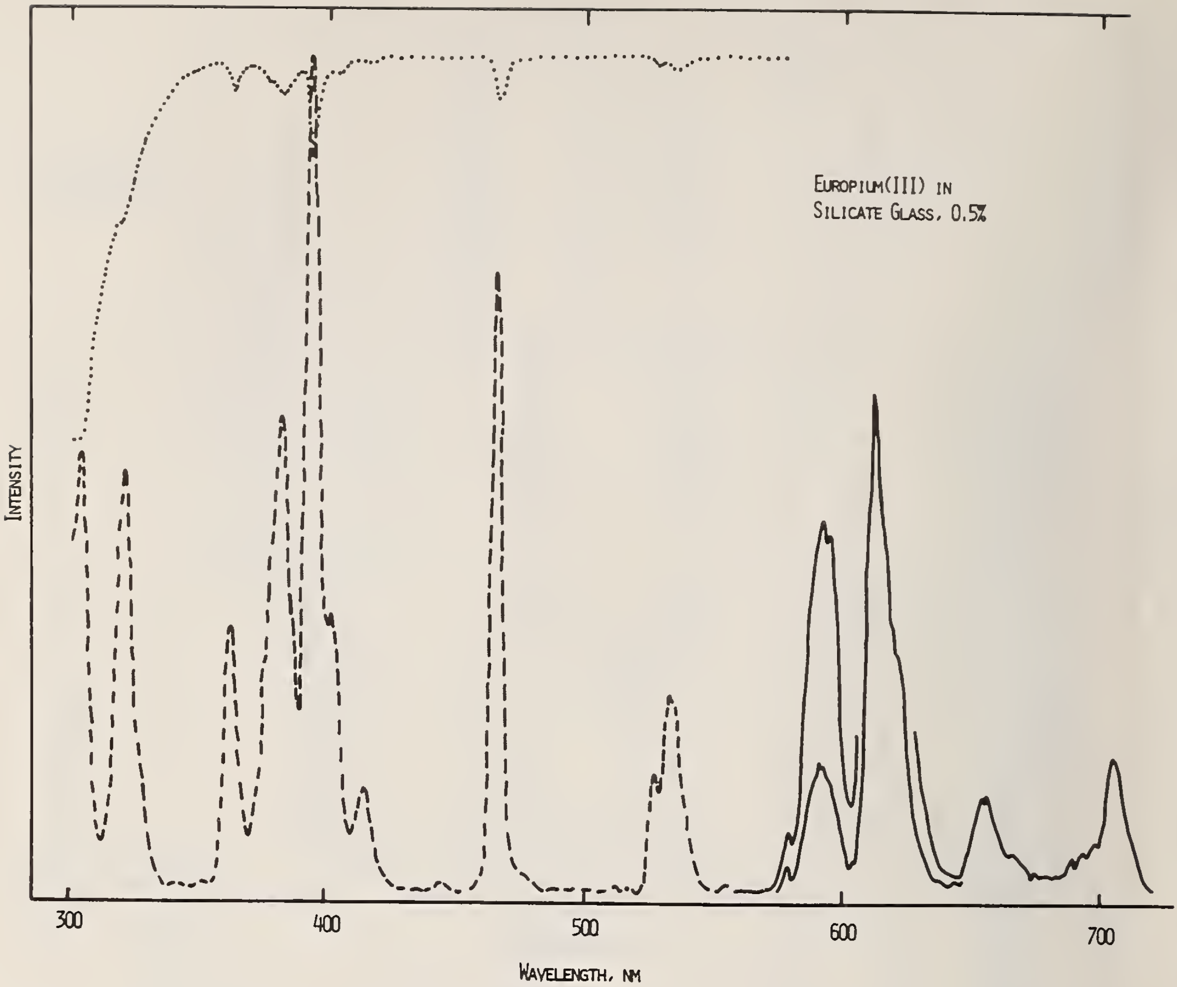


Figure 20. Corrected emission ———, excitation ----- and absorption ..... spectra of europium(III) in sodium silicate glass prepared by the Inorganic Materials Division at NBS.

University in Israel, who, in conjunction with NBS, is developing the spectrofluorimetric chemistry of lanthanide glasses. The Eu(III) in silicate glass was obtained from the Inorganic Glass Section of the Inorganic Materials Division at NBS. Very similar optical properties exist between the two glasses. As found by Dr. Reisfeld, definite wavelength shifts occur for some of the band maxima due to matrix effects. The emission maxima are relatively sharp.

Two other glasses, however, give emission spectra which are fairly broad, figures 21 and 22: 75 nm half-band

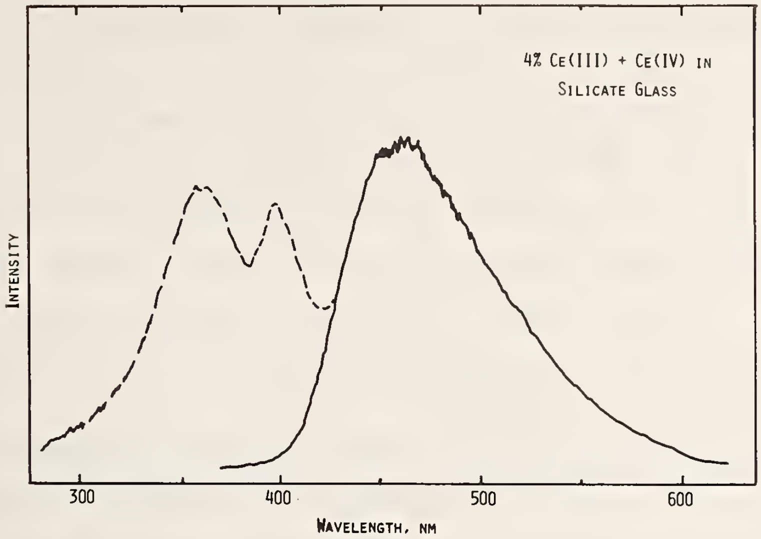


Figure 21. Uncorrected emission ——— and excitation ----- spectra of a 4% cerium(III) - cerium(IV) mixture in silicate glass obtained from the Inorganic Materials Division at NBS.

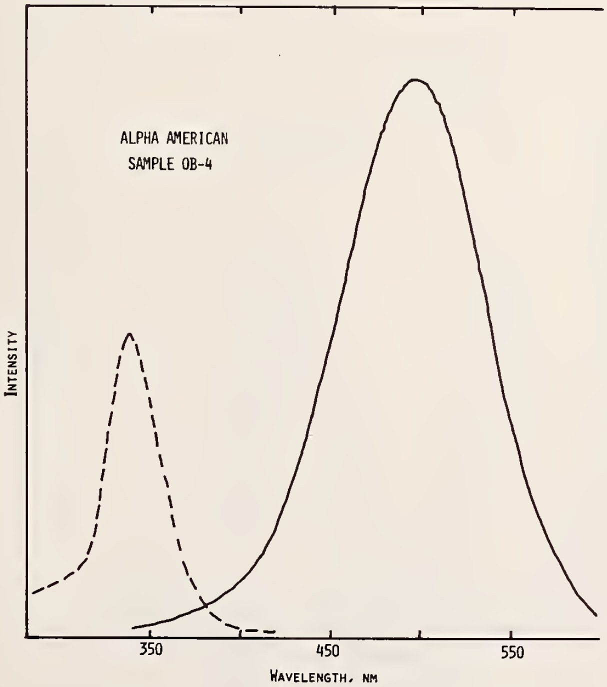


Figure 22. Uncorrected emission ——— and excitation ----- spectra of sample OB-4 fluorescent glass obtained from Alpha American, Rockford, Illinois.

width height for the 4 percent Ce(III) - Ce(IV) in a silicate sample obtained from the Inorganic Glass Section of the Inorganic Materials Division and 110 nm half-band width height for the Alpha American OB-4 sample. These two spectra were taken on the uncorrected spectrofluorimeter because of high inorganic salt concentration. These spectra may be distorted due to inner filter effects. Nevertheless, these two glasses look very promising as SRM's and further developmental work is planned.

Other glasses incorporating Eu(II), and techniques such as the addition of organolanthanides or chelates to glasses may produce broader emission spectra due to $\pi^* \rightarrow f$ electronic transitions (rather than narrow $f^* \rightarrow f$).

Figure 23 presents spectra of uranium in silicate glass showing a broad emission spectrum. The only sample available was one containing 15 percent uranium oxide which is too concentrated, resulting again in inner filter and

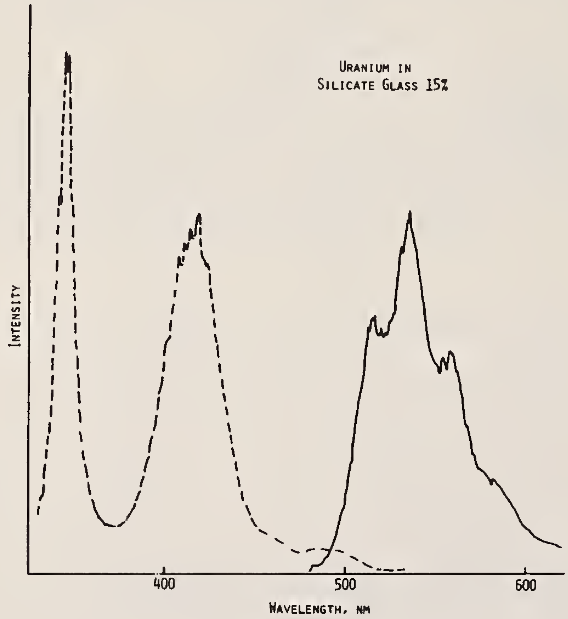


Figure 23. Uncorrected emission ——— and excitation ----- spectra of 15% uranium in silicate glass fluorescent standard used by Carl Zeiss, Inc.

quenching effects which were discussed in connection with Ce spectra [23].

The final SRM spectra will be published with the following information: Q_f, emission intensities in absolute energy units, inorganic salt concentrations, glass stabilities, corrected spectra versus wave number and methodology for attaining best results.

Corrected spectra will be published in subsequent NBS circulars. Several typical spectra are presented in figures 24-26, fluorescein, pyrene and mesoporphyrin IX dimethyl ester. Figure 24 is of fluorescein, which is used for tracing flows of water and sewage in bays, streams and sewage systems.

Pyrene, spectra given in figure 25, is one of a group of polynuclear aromatic hydrocarbons, some of which are carcinogens. They are in polluted air and lend themselves to sensitive spectrofluorimetric determinations.

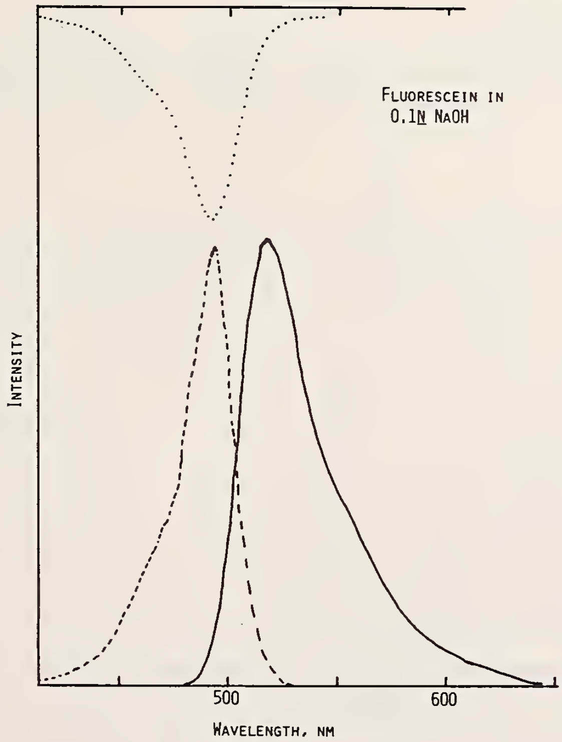


Figure 24. Corrected emission ———, excitation ----- and absorption ..... spectra of 10 ppm fluorescein in 0.1N NaOH.

Figure 26 presents the spectra for mesoporphyrin IX dimethyl ester which shows the usefulness of fluorescence in clinical and biological chemistry. The analytical determinations of porphyrins, as well as numerous other biologically important species, involve spectrofluorimetry. In future work, these corrected spectra will be produced by feeding data from the spectrofluorimeter directly into a computer for point by point plotting of intensity versus wave number (rather than wavelength as presented here). Electronic integration of the emission spectra and comparison of known values of a developed standard will give comparative Q_f values. All necessary data will be included in these circulars such as concentrations, solvents, temperature dependence, quenching effects and statistical data analyses.

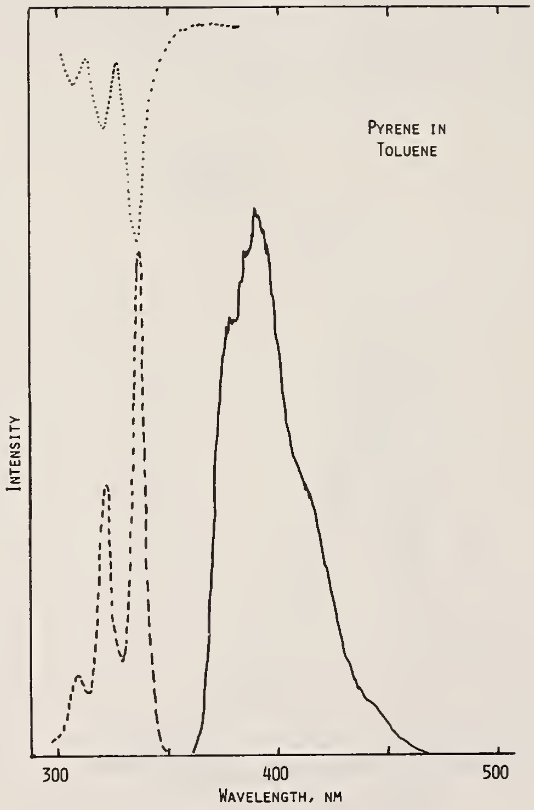


Figure 25. Corrected emission ———, excitation ----- and absorption ..... spectra of 5 ppm pyrene in toluene.

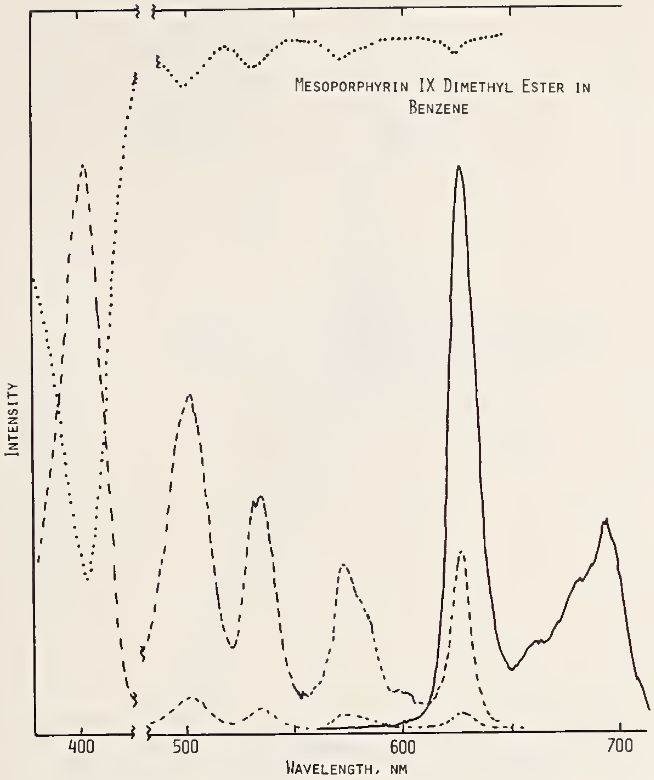


Figure 26. Corrected emission ———, excitation ----- and absorption ..... spectra of 10 ppm mesoporphyrin IX dimethyl ester in benzene.

C. Study of the Rhodamine 6G Fluorescence; Importance of Having Corrected Spectra (R. W. Burke and R. A. Velapoldi)

It is widely accepted that fluorescence spectra are needed which do not include instrumental artifacts [20]. A graphic illustration of this need is presented. Rhodamine 6G in ethanol gives an uncorrected excitation spectrum, figure 27. Sharp peaks are observed on the short wavelength side of the excitation band which could erroneously be assigned to electronically produced fine structure.

The xenon source has an emission spectrum (determined by placing an aluminum block cut at a 45° angle in the sample holder) which is also presented in figure 27. Extremely sharp bands are noted, some of which correspond to the 'fine structure' for the Rhodamine 6G. Substitution of a

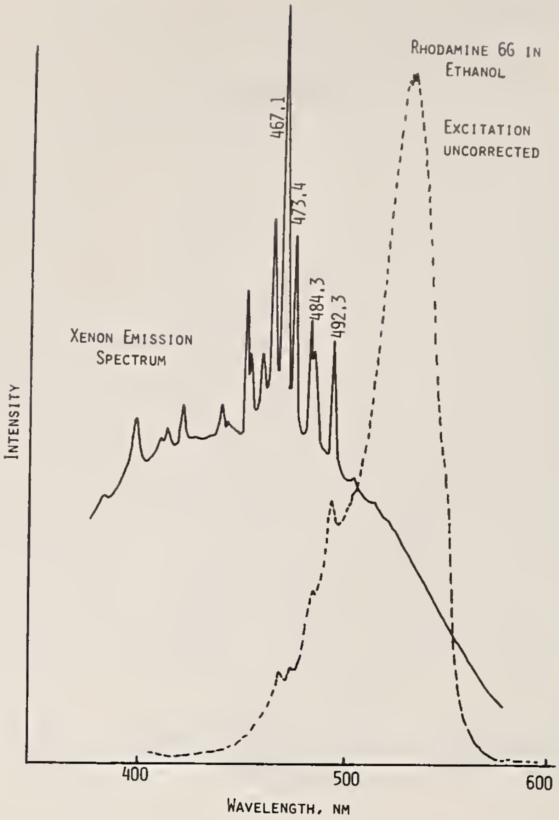


Figure 27. Xenon source emission spectrum ——— and the uncorrected excitation spectrum ----- for 3 ppm Rhodamine 6G in ethanol.

tungsten iodide lamp for the xenon source eliminates these sharp peaks of the excitation spectrum which now shows only the presence of a shoulder.

Figure 28 presents spectra taken on a corrected spectrofluorimeter operating in the corrected and uncorrected modes and also summarizes earlier observations. The uncorrected excitation spectrum is not the same as the absorbance spectrum. The corrected excitation spectrum has the same shape as the absorbance spectrum and occurs at the same wavelength. It is not hypsochromically shifted by 5 nm as is the uncorrected excitation spectrum. There appear to be no differences between the corrected and uncorrected emission spectra, although measurements of Q_f's must be made as proof.

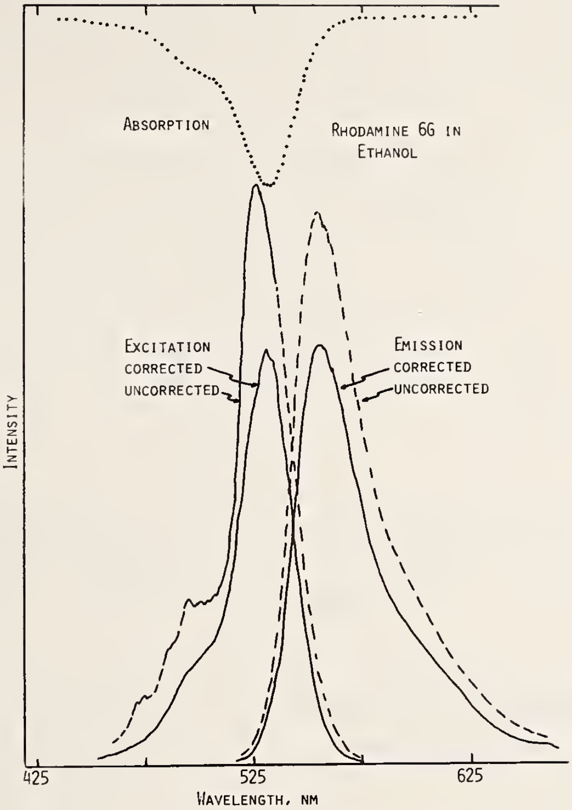


Figure 28. Summary of corrected and uncorrected emission and excitation spectra and the corrected absorption spectra for 3 ppm Rhodamine 6G in ethanol.

This is definite evidence, then, of confusion which results by the publication of uncorrected spectra and underscores the need for a corrected spectra publication.

D. Fluorescence and Absorption of Rare-Earths in Glasses

(R. Reisfeld, Hebrew University).

In an effort to find suitable standards for fluorescence (and perhaps for absorption spectrophotometry), we concentrated on the incorporation of rare-earth ions into glass matrices which fluoresce in the ultraviolet and visible part of the spectrum [24]. The energy transfer between Sm(III) and Eu(III) was studied in order to find the mutual influence of these ions on their fluorescence [25]. The determination of absolute quantum yields for these rare earth glasses could lead to the development of reference standards in the spectral regions of interest. One factor in the production of standards is the prerequisite that the emission spectrum of the standard should have a wide half band width (100-150 nm).

While many of the rare-earths have narrow emission band widths, some, including cerium(III) have wide band widths. The fluorescence of Ce(III) is attributed to the transition of the electron from the 5d orbital to the $^2F_{5/2}$ level. The overlap of the 5d level with the surrounding ligand fields is rather strong, explaining the great width of the emission band.

The experimental results of measurements made on cerium-doped glasses are presented here. The theory is currently being developed and it is planned to publish these results in the near future.

1. Cerium in Silicate Glasses

The glasses examined were of the following base composition: SiO_2 - 72%; Al_2O_3 - 1.5%; CaO - 7.5%; MgO - 4%; Na_2O - 15%. CeO_2, Fe_2O_3 and in some cases F^- were added to the base glass in the following concentration ranges: CeO_2 - 0.007 to 0.3%; Fe_2O_3 - 0.021 to 0.093% and F^- - 0.5 to 1%. Addition of iron serves to reduce Ce(IV) to Ce(III):

$$\mathrm{Ce(IV)} + \mathrm{Fe(II)} \rightleftharpoons \mathrm{Ce(III)} + \mathrm{Fe(III)}$$

The absorption spectrum of Ce(III) in silicate glass versus the base glass is presented in figure 29. The excitation (emission at 385 nm) and emission (excitation at 330 nm) spectra of Ce(III) are given in figure 30. Qualitative data show that the relative fluorescence is cerium concentration dependent. The relative fluorescence increases with cerium concentration, e.g. a 32.4% increase is produced by raising [Ce(III)] from 0.1% to 0.2% and 51.0% by increasing the [Ce(III)] from 0.1% to 0.3%. Increasing the Fe_2O_3 concentration results in a slight enhancement (7.3%) of the relative fluorescence. Fluoride

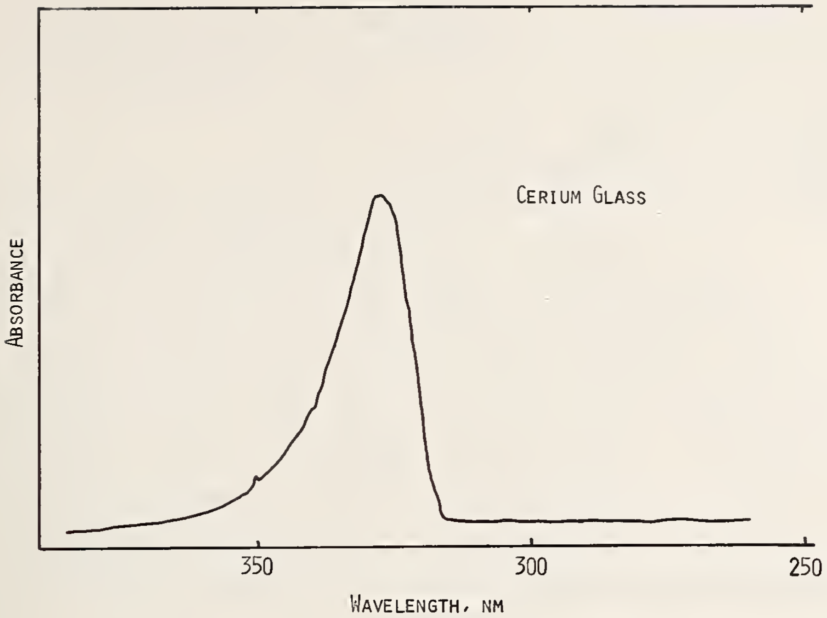


Figure 29. Absorption spectrum of cerium(III) in silicate base glass run versus silicate base glass reference.

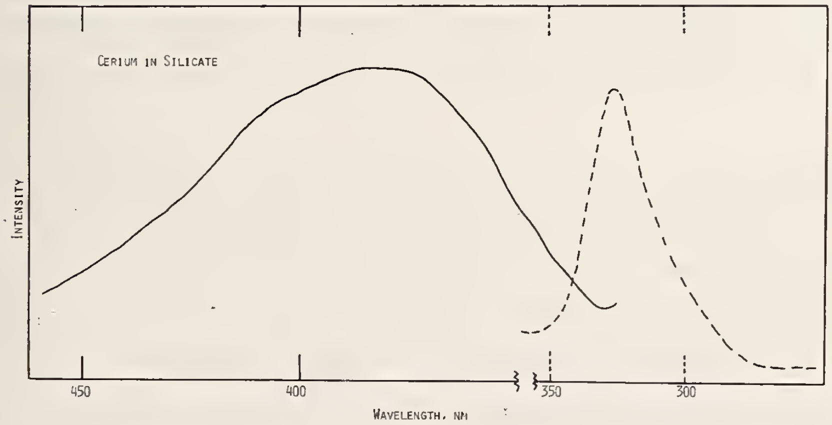


Figure 30. Excitation spectrum ----- (emission at 385 nm) and emission spectrum ——— (excitation at 330 nm) of cerium(III) in silicate glass.

addition increases the fluorescence slightly probably due to a shift in equilibrium of iron oxidation. Equilibrium constants of this reaction may be computed and will be done in the future.

These glasses will be subjected to varying periods of ultraviolet irradiation in order to test the reproducibility and stability with time.

2. Cerium in Borate Glasses

The basic constituents of these glasses were borax and boric acid in a 2:1 ratio. The detailed procedures for glass preparation are described in earlier publications [26]. Commercial $CeCl_3 \cdot 7H_2O$ was used for the preparation of the Ce(III) glasses and CeO_2 was used for the preparation of the Ce(IV) glasses. Absorption spectra of the glasses were measured in order to determine the distribution of Ce(III) and Ce(IV) and the best absorption band for excitation. All glasses showed an absorption band extending from 220 to 320 nm. This large band is clearly an overlapping of the Ce(IV) (charge transfer) peaking at approximately 250 nm and Ce(III) ($f \rightarrow d$ transition) peaking at approximately 315 nm. Regardless of the starting cerium salt, the cerium containing glasses showed absorption spectra characteristic of a Ce(III) and Ce(IV) equilibrium. Preparation of cerium glasses in a reducing atmosphere to insure a preponderance of Ce(III) is planned.

The excitation (350 nm emission) and emission (315 nm excitation) spectra are presented in figure 31. The excitation and emission maxima of the cerium couple in borate glasses occur at shorter wavelengths than in the silicate glasses, probably due to varying crystal fields exhibited by the different matrices, substantiating extensive overlap between the 5d orbital and the matrix. It is hoped that by producing a matrix of appropriate silicate to borate mixtures, an emission band with greater halfwidth will be obtained. The influence of the exciting wavelength on the fluorescence will also be studied.

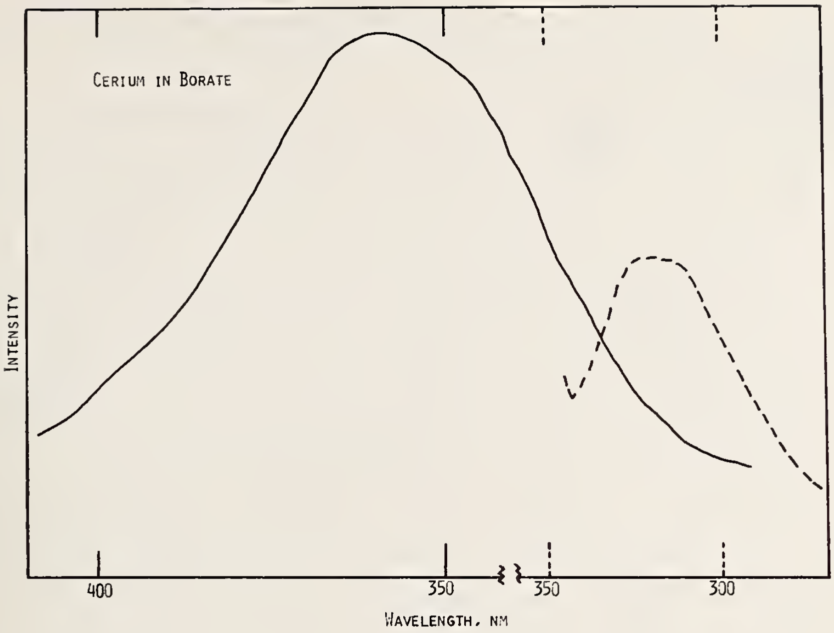


Figure 31. Excitation spectrum ----- (emission at 350 nm) and emission spectrum ——— (excitation at 315 nm of a cerium(III) - cerium(IV) borate glass.

3. ANALYTICAL FLAME SPECTROSCOPY

A. Introduction

The recognized importance of flame spectrometry as an analytical technique is evident from the new review in Analytical Chemistry [27]. This reflects the increasing research in this field as well as its wide application to the analytical needs for relatively simple, very rapid and precise methods. This growth is not limited to atomic absorption, but includes the revived interest in flame emission work and in expanded efforts in atomic fluorescence. It has been shown that as little as 10^{-14}g of an element can be detected in several milligrams of sample. Major constituents, however, can be determined with a relative standard deviation of 0.2 percent [28].

In a continued effort to develop this competence, instruments of new design are being tested; components and flame data are being evaluated. This section describes the progress in instrumental innovations, various flame components, studies of relative resonance and non-resonance line intensities, and special applications to Standard Reference Materials.

B. Multipurpose Precision Flame Spectrophotometer

(R. Mavrodineanu)

The program established in this field was directed toward the design and construction of a precise flexible flame spectrophotometer capable of performing measurements in the flame emission, atomic absorption, and flame fluorescence modes. Further requirements were that single and simultaneous multielement measurements be possible with an electronic integration of the optical signals, and with data presentation in analog (recorder) form and simultaneous digital print-out, punch-out form.

1. Design of the Instrument

a. Optical System. The instrument was designed and built along a one meter grating spectrometer using the Czerny-Turner optical arrangement and capable of functioning in the scanning mode (variable exit slit), photographic mode (2" x 8" plate holder), and simultaneous multichannel arrangement (compartment accepting up to 18 side-on photomultiplier tubes and a multiple slit-carrying plate). A rigid 160 cm long lathe bed-type optical bench was placed along the optical axis in front of the entrance slit of the spectrometer, as illustrated in figures 32 and 33. The following equipment is assembled on the optical bench using appropriate carriers: a quartz lens which produces a demagnified image of the analytical flame on the entrance slit; the burner and sprayer unit with its dual sample supplying system; a second quartz lens which produces a demagnified image of the hollow cathode in the flame; a mirror chopper which generates an ac signal and at the same time combines in one beam the emission from the two hollow cathodes in succession along the optical axis. The lenses, flame, chopper and hollow cathodes can be positioned with precision along the optical axis in the x-y-z direction and tightly secured in the correct location (see figures 32 and 33).

b. Electronic Circuitry. The electronic equipment with the scanning mode is shown in figure 32. It consists of a differential preamplifier capable of handling separately two signals, A or B, as well as presenting their difference A-B. This preamplifier is followed by a selective amplifier, a lock-in amplifier, an analog to digital converter with digital presentation and a recorder. The power supply for the hollow cathodes is also shown in the figure and consists of a current stabilized unit ($\leqq \pm 0.1\%$). The electronic circuitry used with the multichannel mode

Figure 32. Flame spectrometer.
Left - console with the electronic measurement system for the scanning mode system, and power supply for the hollow cathode.
Middle - one meter spectrometer and sprayer and burner unit producing a premixed air-acetylene flame. The pneumatic sprayer is supplied with the analytical solution through a two way Teflon stopcock.
Right - water pressure gauge for monitoring the pressure in the spraying vessel and burner unit.

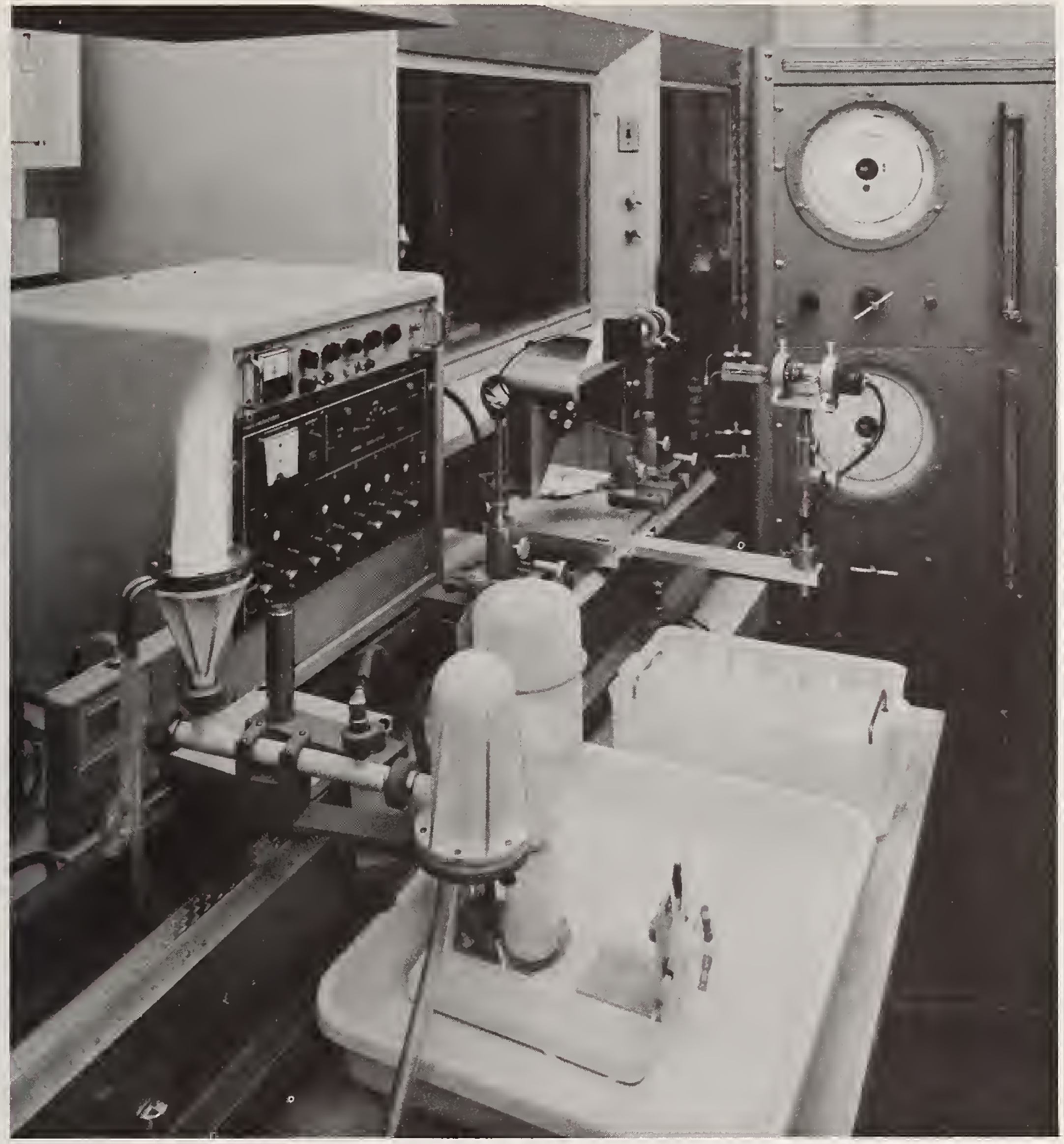


Figure 33. Flame spectrometer.
Left - optical bench carrying the sprayer and burner unit.
Middle - electronic unit for the multichannel operation.
Right - mirror chopper, dual hollow cathode arrangement, and gas regulating and monitoring console.

was available from previous work [29] and is shown in figure 33. The same figure shows the console which carries the gas regulating and monitoring system for

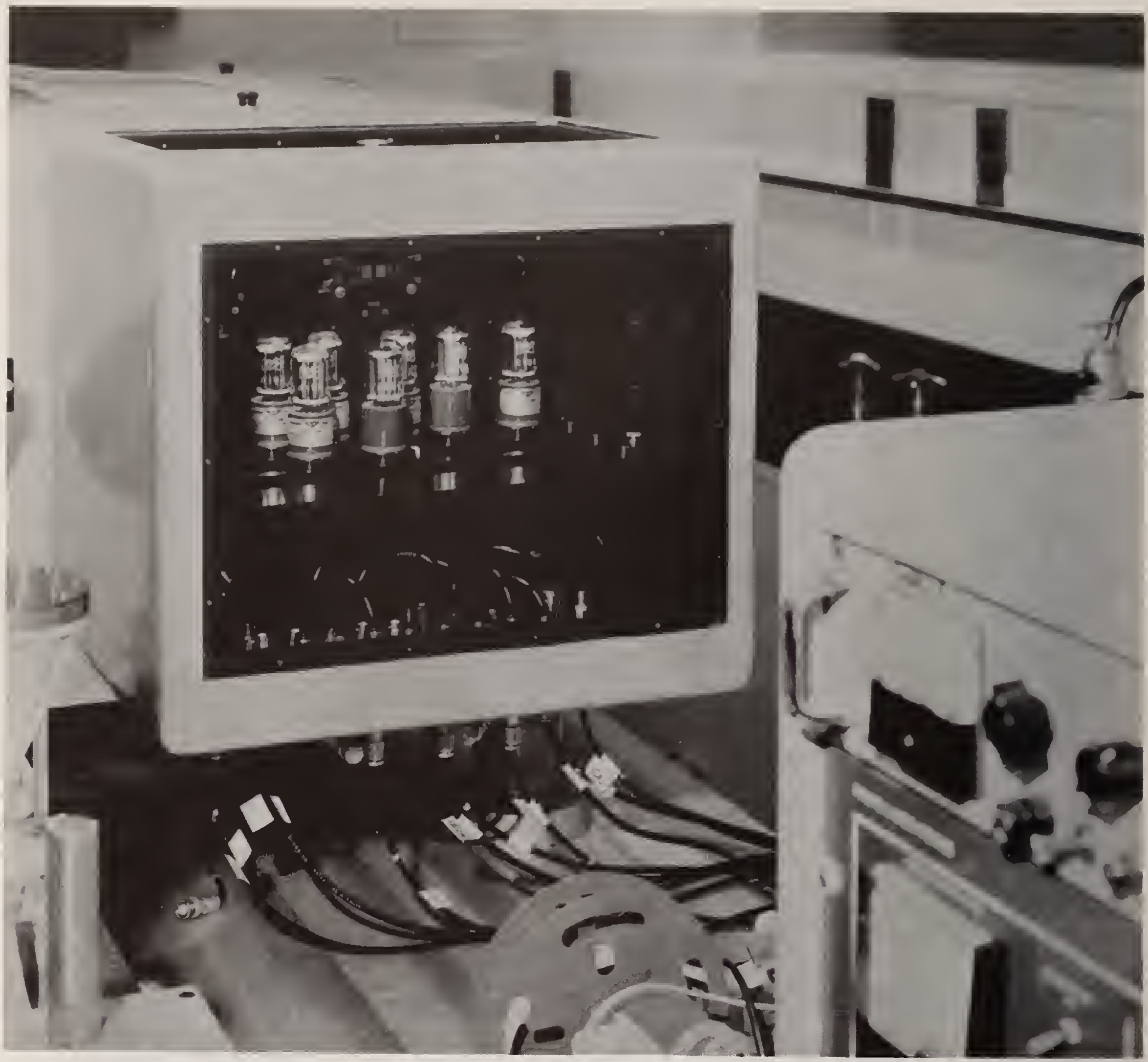


Figure 34. Flame spectrometer.
Inside view of the multichannel photomultiplier arrangement. Seven tubes are disposed behind individual exit slits.

both fuel and oxidant (pressure regulators, high precision gauges, 250 mm flowmeters). Figure 34 illustrates the photomultiplier arrangement in the rear of the multislit carrying plate. Seven tubes are used to receive the optical signals from Na (5893 Å); K (7667 Å); Li (6707 Å); Ca (4227 Å); CaOH (6230 Å); and Mg (2852 Å); the seventh tube is used in conjunction with the scanning mode. The first 6 photo-

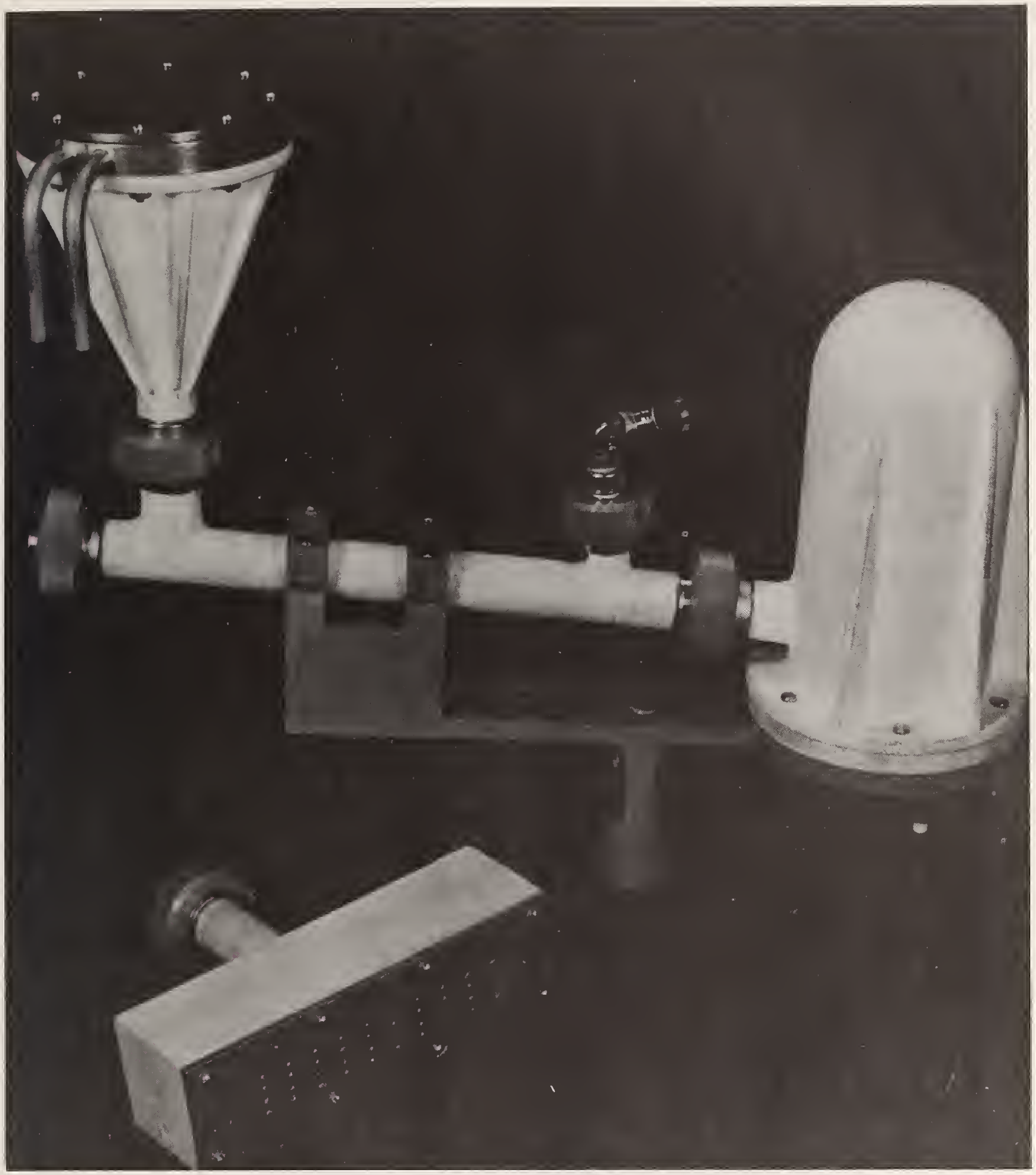


Figure 35. Sprayer and burner unit. An interchangeable burner head is seen at the bottom of the figure.

multiplier tubes are connected to the multichannel electronic system, (figure 33), while the seventh tube is connected to the scanning electronics shown in figure 32.

c. Burner and Sprayer System. The burner and sprayer system was available from previous work [30] and is shown in more detail in figure 35. It consists of a pneumatic sprayer spraying vessel and burner body made of alumina (99 percent purity), and of a water cooled burner plate made of tantalum. This unit is corrosion and erosion resistant and flame proof. The gas mixtures which can be used with this flame are: air-acetylene, nitrous oxide-acetylene; nitrous oxide-propane.

The spraying vessel is connected to a drain bottle through a glass hydraulic trap and is illustrated in figure 36 (available from a previous work; see reference 30).

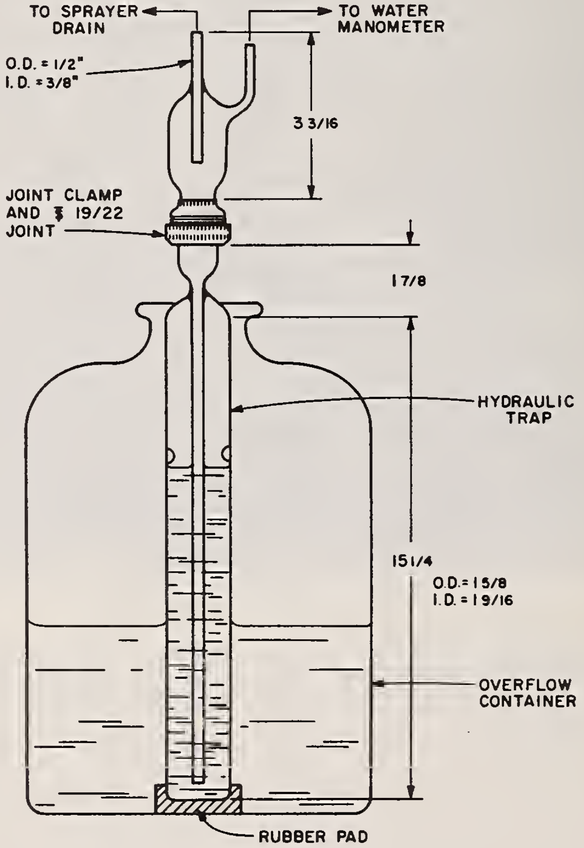


Figure 36. Hydraulic trap made of glass.

The functioning of the spectrometer in the scanning mode is conventional and does not require further discussion. (Since the electronic measuring system for the scanning mode uses a lock-in amplifier, a mechanical chopper is placed between the emitting flame and the entrance slit of the spectrometer.) The multielement mode, with simultaneous flame emission and atomic absorption measurements, will be described briefly since it is a less conventional operation.

The heart of the system is the electronic measuring and data displaying unit (figure 33). This unit has a capacity of 8 channels. Two channels and the electronic design are illustrated in the block diagram in figure 37a. The signal from each photomultiplier tube (PM) is fed to individual integrating capacitors where it is stored and then sequentially converted to measurement in digital form. The digital readout is displayed by a printer and paper tape puncher. In the latter, the data are compatible from direct input to an IBM 1130 computer. The light measuring channels

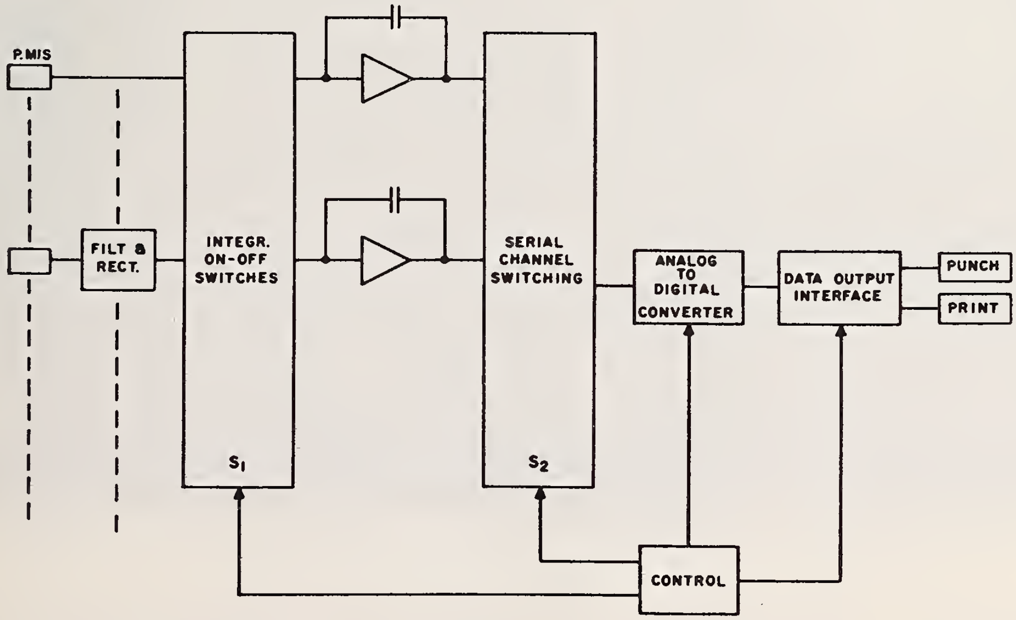


Figure 37a. Block diagram of the electronic system used in conjunction with the multichannel spectrometer.

can be used to accept either ac or dc inputs in any combination. The former is used in conjunction with the flame atomic absorption mode, while the latter is used for the emission mode (see reference 29). Every channel has a sensitivity adjustment and a dark current compensation capability. The integration time can be selected stepwise as needed from 10, 20, 40, 100, 200, 400, and 1000 seconds. The measurements can be made in 1, 2, 3 or 4 replications or continuously, the sequence in this case being interrupted manually (figure 37b). The simultaneous digital printed or punched-out display contains the following information:

Figure 37b. Front panel of the electronic system shown in 37a. From bottom to top -- dark current compensation, channel sensitivity selectors, mode of operation switches, power supply for photomultiplier tubes.

channel number, sensitivity setting, integration time, and the arbitrary counts corresponding to the charge of the individual capacitors. The arbitrary counts are then converted to meaningful information through calibration with analytical solutions containing known amounts of analyte.

In the present configuration, the electronic system is set to accept four signals in the dc mode for flame emission measurements of Ca (CaOH) K, Na, and Li which is used as an internal standard, and four signals in the ac mode for measurements by atomic absorption techniques. From these four ac channels, only two are used in the present arrangement to determine Mg and Ca using the mirror chopper at 45° on the optical axis, and using two hollow cathodes (figure 33). If a two-element Mg-Ca hollow cathode tube is used, then the chopper equipped with a normal blade is placed at 90° on the optical axis.

2. Experimental Tests

To determine a suitable chopping frequency, both in the emission and atomic absorption mode, a series of measurements was designed and performed to determine whether the analytical flame itself exhibits any periodicity in its radiation which could interfere with the chopper frequency. These measurements were made by an oscilloscope in cooperation with Mr. F. E. Ruegg, Analytical Chemistry Division, Technical Services. The results are shown in figure 38 where it can be seen that the unsupplied flame exhibits a "white noise" type of periodicity of low amplitude. When an element, such as Na, was supplied to the flame, the resulting signal indicated a strong periodicity at a frequency of 25 Hz. As a result of these measurements, the chopping frequency for the measurements in the ac mode was selected to avoid interferences with the fundamental frequency of the flame or any of its harmonics.

The scanning electronic system was tested to determine its general functioning conditions by using the single

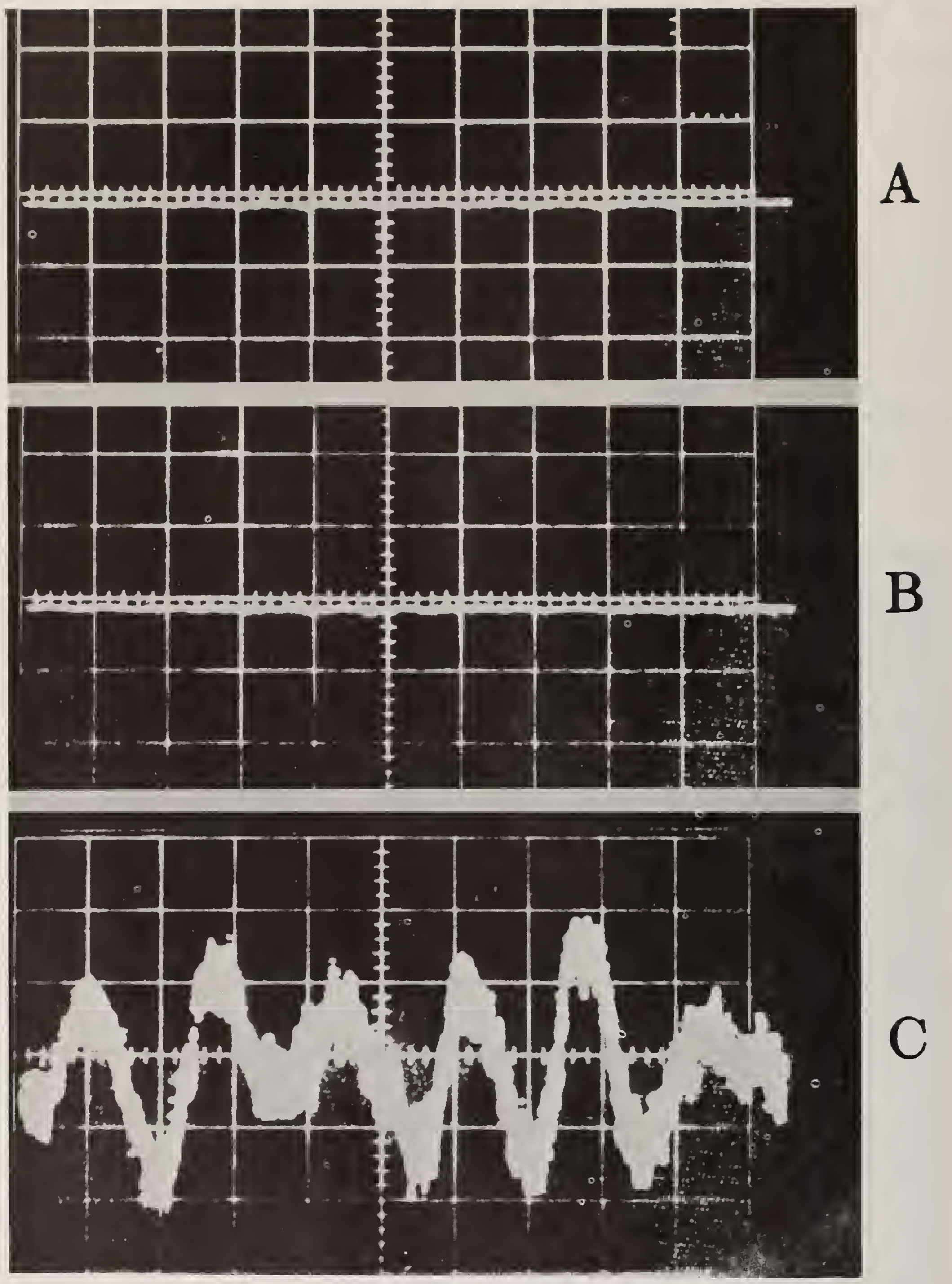


Figure 38. Flame fluctuation as illustrated on an oscilloscope screen. A — signal produced by an unsupplied premixed air-acetylene flame at λ 5893 Å. B -- signal produced by the same flame supplied with distilled water at λ 5893 Å. C -- signal produced by the same flame supplied with Na salt at λ 5893 Å.

signals (A or B) from Na and Li as the differential A-B signal. In this latter mode, the signal from B can be produced by the radiation from a constant concentration of Li, while A can be produced by a variable concentration of Na. The differential signal A-B will then, to some extent, represent an internal standard operation and should result in an improved stability of signal A (Na). The results obtained under these conditions indicate that when using the sprayer and burner unit (figure 35), a reduction of the "noise" of signal A by a factor of 3 was observed.

Further evaluation of the electronic systems for their sensitivity, stability and reproducibility will be made by using a constant source of radiation in the form of a fluorescent material excited by tritium (half-life 12.5 years). The flame emission and atomic absorption spectrometer described earlier will then be used for measurements in conjunction with clinical analytical problems.

C. Long-path Flames (R. Mavrodineanu)

In addition to the sprayer and burner unit shown in figure 35, an experimental unit was designed and constructed to produce a premixed acetylene—air flame having a long path. This prototype unit, made of glass and copper is illustrated in figure 39. The flame produced with this burner is 30 cm long and 1.0 cm wide. The all glass external pneumatic sprayer supplies the flame with the analytical sample at the rate of about 0.34g/min for a sample consumption of 18 ml/min. The evaluation of this unit is now in progress.

D. Hollow Cathode After Grimm (R. Mavrodineanu)

In 1968 Grimm gave a detailed description of a glow discharge unit which was capable of operating as a flat discharge or as a hollow cathode discharge [31]. It is well known that hollow cathode discharges are particularly suitable for the excitation of small amounts of many chemical species with great sensitivity [32]. Hence, it was considered desirable to test the detection capabilities of such an

Figure 39. Long-path flame 30 cm long and 1.0 cm wide produced by the premixed combustion of air with acetylene. Experimental prototype unit.

excitation source in conjunction with chemical species of particular interest to the clinical chemist. A Grimm hollow cathode discharge unit was available and was set-up in conjunction with the 1 m grating spectrometer discussed previously; figure 40 illustrates the arrangement. Pure copper hollow cathodes were supplied with varying amounts of Mg in aqueous solution using a volume between 3 and 10 μl containing 0.3 to 5 ng Mg. The transfer of the sample was made under a microscope using micromanipulator

Figure 40. Hollow cathode discharge unit after Grimm. The hollow cathode is located in the enclosure placed on the optical bench. The power supply and various controls are located in the console at the extremity of the optical bench.

techniques. The spectra were recorded photographically for an exposure time of 20 sec using a 1200 line/mm grating in the second order. The argon pressure was 1 Torr and the current supplied to the hollow cathode unit was 200 mA. Under these experimental conditions the detection limit for Mg was 0.3 ng.

E. Spectrophotometric Instrument for Ratios and Difference Measurements (T. C. Rains)

An instrument capable of measuring the ratio or difference in two signals has been designed, built and is being evaluated. In the absorption mode, variations produced by the source can be cancelled out by taking the ratio of the light flux coming from the absorption cell and from the source directly. In flame emission, the problem is one of separating the background radiation from the signal produced by the radiant intensity of the analytical line. The isolation of the radiant intensity of the analytical line is accomplished by subtracting the common background radiation.

The instrument consists of a 0.5m and a 0.25m monochromator, light-beam-chopper disk, beam splitter, dc power supply, lock-in amplifier and digital voltmeter. The lock-in amplifier and digital voltmeter were constructed by the Measurement Engineer Division of the National Bureau of Standards. It contains two identical channels, one for the signal and the other as a reference for obtaining the ratio or difference. Each channel derives its input from a multiplier phototube which is powered by a single dc power source. The output from the photodetector is amplified and fed into a digital readout. In the absorption mode, the outputs of two filter-amplifier stages provide the two potentials for the ratio measurement. In the emission mode the two outputs are combined at a summing junction of resistors, if algebraic differences are desired. The voltage output of this operational stage is the difference signal which is fed to the digital readout.

The instrument is presently being tested for flame emission and atomic absorption spectrometry under various operating conditions.

F. Selection of the Analytical Line in Atomic Absorption

(T. C. Rains and T. A. Rush)

The selection of the appropriate resonance line for atomic absorption spectrometry is often made empirically, and this information may often be lacking. The analyst more often seeks the most sensitive analytical line for a given determination and assumes that the line used by other workers, or the one listed by the manufacturer, produces the highest sensitivity. For example, the resonance line of lead at 2170 Å is 2.5 times as sensitive as the line at 2833 Å. However, the latter is usually recommended because the line at 2170 Å is subject to spectral interference from copper, especially if a monochromator of poor dispersion is used. Occasionally a less sensitive line is desirable to extend the dynamic range of the analysis to higher concentrations without having to dilute the sample. For most elements, the analytical line is generally the resonance line with the largest oscillator strength corresponding to the transition from the ground state to some higher excited state. If the ground state is a multiplet with only slightly different energies, then the number of atoms occupying the higher energy levels may be greater than those in the lower energy level, and thus provide higher sensitivity. For aluminum, the ground state (0 eV) resonance line at 3082 Å has a gf (product of statistical weight, g, and oscillator strength, f) of 0.38. Although the lower energy level line at 3093 Å is 112 kaysers or 0.014 eV, with a gf value of 0.79; it is twice as sensitive as the 3082 Å line. A comparison of the sensitivity of the Al I lines using a fuel rich nitrous oxide—acetylene flame is given in table 11.

If the gf values could be accurately established, the sensitivity of the major analytical lines could be readily

Table 11. Relative sensitivity of Al I lines in atomic absorption.

(Oxidant-fuel: N_2O - C_2H_2 (fuel rich))

Wavelength, Å	gf	Sensitivity µg/ml/% abs.
3092.7	0.79	2.1
3961.5	.31	3.0
3082.2	.38	4.0
3944.0	.15	5.2
2373.1 } 2373.4 }	.31 } .061 }	5.5
2667.1	.15	8
2269.1	.092	10
2567.9	.10	16
2378.4	.015	50

calculated. This can be shown from the relationship of the oscillator strength of an analytical line to the Lambert-Beer Law in the following manner. The Lambert-Beer Law states that the absorbance of an analytical line is proportional to the absorptivity, a, of the analyte sprayed into the flame, to the concentration, c, of the absorbing atoms and to the length of the absorption cell, b, and is given by

$$A = 0.4343\ a\ b\ c$$

If the absorbance is measured over the entire width of the absorption line, a is equal to $f_{ji} \frac{\pi\varepsilon^2}{mc}$ where f_{ji} is the oscillator strength for the transition involving the upper level, j, and the lower level, i, ε is the charge of the electron, m is the mass of the electron and c is velocity of light [33]. If the lower energy level is the ground state, then c is simply related to the number of atoms in the ground state N_o. If energy levels above the ground state are involved in the absorption of energy, then the number of atoms in the excited state, i, is given by the Boltzman expression

$$N_i = N_o \frac{g_i}{g_o} e^{-E_i/kT}$$

The Lambert-Beer law can be rewritten in the following form

$$A = 0.4343 \frac{\pi\varepsilon^2}{mc} \frac{N_o}{g_o} g_i f_{ji} e^{-E_i/kT}$$

or simplified to

$$A = K \frac{N_o}{g_o} g_i f_{ji} e^{-E_i/kT}$$

For ground state resonance lines, the expression $e^{-E_i/kT}$ would be equal to one. Therefore, the absorbance, A, is proportional to the statistical weight factor, g, and the oscillator strength, f.

Table 12 gives a listing of the most useful Cr I lines that have analytical applications in atomic absorption. The most sensitive line using a fuel rich air–acetylene flame is 3578.7 Å. A typical example of the usefulness of this information can be noted from the data shown in figure 41. From a calibration curve of 0 to 5 µg/ml of chromium, a linear relationship is obtained with a maximum

Table 12. Relative sensitivity of Cr I lines in atomic absorption

(Oxidant-fuel: N_2O - C_2H_2 (fuel rich))

Wavelength, Å	gf	Sensitivity µg/ml/% abs.
3578.7	1.6	0.22
3593.5	1.4	.29
3605.3	1.0	.35
4254.4	0.54	.55
4274.8	.41	.77
4289.7	.26	1.1
3615.6	.0069	20
2364.7	.033	45

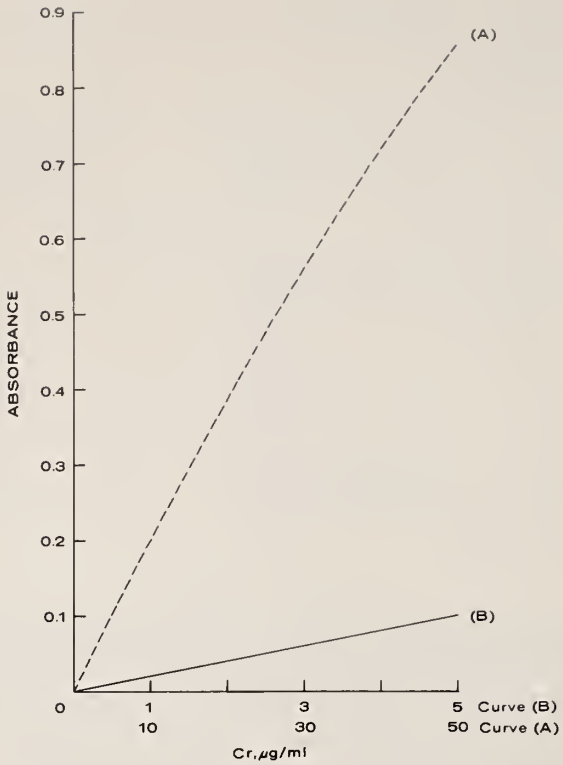


Figure 41. Calibration curves for the chromium line at 3579 Å.

absorbance of 0.1. By working with concentrations of 0 to 50 µg/ml of chromium, the curve is extended to 0.9 absorbance units. These absorbance values can be extended to higher values by using scale expansion. On the other hand, if one desired to work with concentrations of 0 to 500 µg/ml of chromium, the length of the optical path of the flame cell would have to be reduced or a less sensitive chromium line could be selected. The choice of line would depend upon the concentration of chromium. Table 12 could aid the analyst in the selection of the appropriate line. Typical calibration curves are shown in figure 42 for the chromium line at 4290 Å. However, the published gf values are known to be in error, especially for those lines below 2500 Å. A program has been initiated to evaluate the sensitivity of all neutral lines for 50 elements. These data will then be correlated with the best gf values.

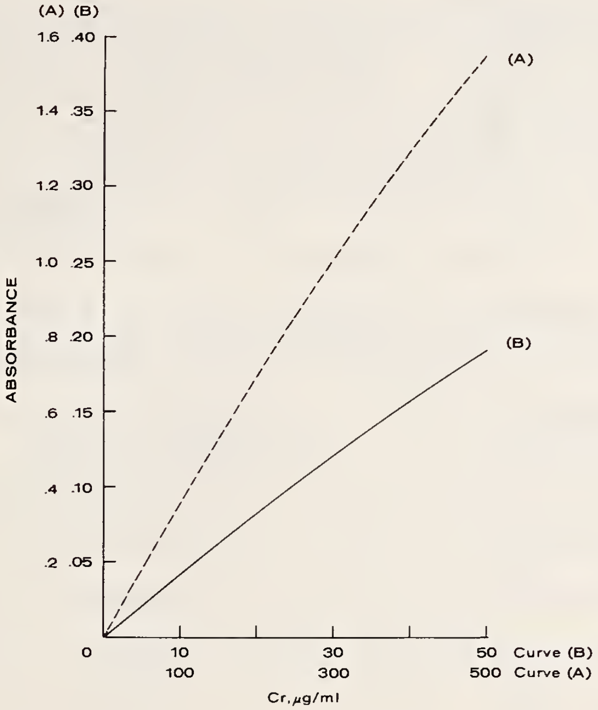


Figure 42. Calibration curves for the chromium line at 4290 Å.

G. Selection of Non—absorbing Line in Atomic Absorption

(T. C. Rains and T. A. Rush)

The application of atomic absorption spectrometry to trace analysis for many elements offers a high degree of precision and a solution of the sample can be aspirated directly without prior separation. However, errors may result from scattering of light from the solid particles within the flame or from molecular absorption when aspirating the sample with a high solid content. To correct for this type of interference, a non-absorbing line must be selected and the absorption signal produced by the sample measured. The absorbance obtained at the non-absorbing line is substracted from the value obtained at the analytical line. The non-absorbing line should be within 100 Å of the analytical line and in some applications the measurement should be made within a few angstroms of the analytical line.

The non-absorbing line can be selected in various ways. With a continuous source, the measurement is made just off the peak of the analytical line. To obtain a non-absorbing line from within the analyte source (hollow—cathode lamp) a scan is made of the spectrum that is produced by the hollow cathode lamp. A typical example is iron, figure 43. The iron spectrum shows the resonance line at 2483 Å and two strong lines at 2488 and 2491 Å. The latter lines are not ground state lines and show no absorbance at concentrations of up to 5000 µg/ml of iron. Therefore, either line can be used to correct for scattering of light or molecular absorption of extraneous ions on iron at 2483 Å. The analyst should always check the selected non-absorbing line with a relatively high concentration of the analyte to verify that absorption of the analyte will not occur at that wavelength. By scanning the spectrum of each hollow—cathode lamp and identifying all emission lines, a wavelength can be selected to correct for light scatter. If no non-absorbing

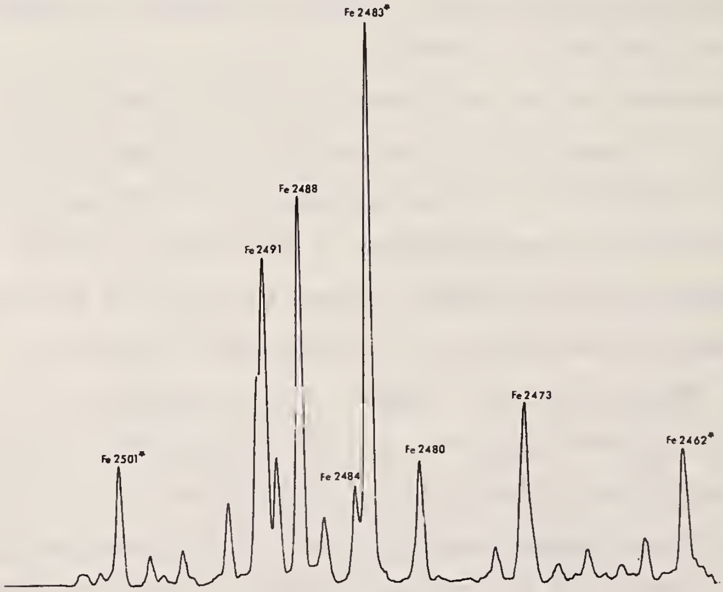


Figure 43. Spectrum of iron over the wavelength region of 2460 Å to 2505 Å.
* - Ground state resonance line.

line is observed within 100 Å of the analytical line, a line from some other light source (hollow-cathode lamp) can be used. The observed height within the flame cell and the slit width, however, must not be changed. Some typical spectral lines, that have been successful in correcting light scattering, are given in table 13.

Table 13. Selection of non-absorbing line to correct for scattering of light or molecular absorption

Analyte	Wavelength, Å	Non-absorbing line Å
Ag	3281	Ne - 3369
As	1937	Ne - 1930
	1972	As - 1990
Ba	5536	Mo - 5533
Mg	2852	Ga - 2874
Pb	2170	Re - 2214
	2833	Ga - 2874
Se	1961	As - 1990
Zn	2139	Re - 2214

H. Phototubes (T. C. Rains)

In a continuing effort to improve our instrumentation for flame emission and atomic absorption spectrometry, two new multiplier phototubes were evaluated. A new S-20 tri-alkali phototube (Centronics 4283) having a spectral response of 2000 to 8500 Å was tested. The tube has a relative low dark current which permits the operation of the tube at voltages up to 1500 V. With the low dark current and high voltage, an improvement in signal-to-noise ratio was obtained for all elements tested by flame emission spectrometry.

For applications in atomic absorption spectrometry, a cesium-tellurium solar-blind phototube (R-166) was tested. With a relatively high resolving monochromator, this phototube offered no advantages over a phototube with a S-5 response; however, in applications of atomic fluorescence where no monochromator is used, the phototube gives an improvement in detection limits for those elements which have resonance lines between 2000 and 3000 Å [34].

I. Shield for Burner (T. C. Rains)

The nitrous oxide–acetylene flame with a temperature of 2955°C is being extensively used in flame emission and atomic absorption spectrometry to increase the atom population for many of the refractory elements and to minimize condensed phase type of interferences. This flame requires a special type of burner head to prevent a flash back and is 18 to 24" in height in its normal mode of operation. Therefore, excessive thermal radiation is produced which may have an adverse effect on the monochromator as well as the operator. To minimize this effect, a heavy shield was designed to fit the burner head. The shield eliminates drift of the wavelength due to changes in ambient temperature in the monochromator and protects the operator.

J. Oxidant–Fuel Mixtures Regulation (T. C. Rains)

The higher temperature and lower burning velocity of the nitrous oxide–acetylene flame eliminates most of the condensed phase interference encountered with the alkaline earth metals. The major disadvantage of the nitrous oxide–acetylene flame is the loss of sensitivity due to ionization of easily excited atoms. To overcome this loss, a gas flow system was constructed and is shown in figure 44. With this system, air and nitrous oxide are mixed to provide a temperature range of 2300°C for the air–acetylene to 2955°C for the nitrous oxide–acetylene flame. In the determination of calcium, magnesium and strontium, a mixture of nitrous oxide and air (80-20) -acetylene under fuel rich conditions eliminates most of the condensed phase type of interferences without loss of sensitivity due to ionization.

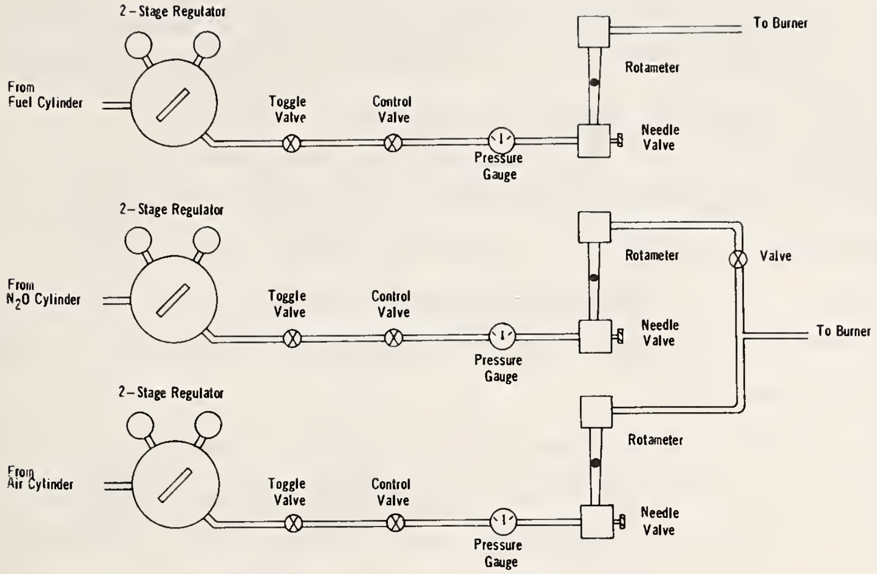


Figure 44. Schematics of oxidant—fuel mixtures regulation.

K. Analytical Application to Standard Reference Materials

(T. C. Rains and T. A. Rush)

1. Determination of Potassium, Rubidium and Strontium in Simulated Lunar Glass

The simulated lunar glasses consist of the base material which is 72 percent SiO_2, 14 percent Na_2O, 12 percent CaO and 2 percent Al_2O_3 and four lots containing 61 elements at the 0.02, 1, 50 and 500 ppm level. The determination of potassium, rubidium and strontium in such a complex matrix became a challenge to the flame photometrist. The first major problem encountered was the dissolution of the sample without contamination. The sample (wafer) was rinsed with ethanol, water and dilute HNO_3 to remove surface contamination. The weighed wafer was then transferred to a platinum dish and ultra pure HF and $HClO_4$ were added. After standing overnight, the sample was heated to strong fumes of perchlorate to complete the dissolution.

Potassium, rubidium and strontium were determined by flame emission spectrometry using the repetitive optical scanner in the derivative mode [35]. The high continuous background produced by the complex matrices, especially at the 50 and 500 ppm levels, prohibits the use of the normal flame emission mode. However, by the technique of repetitive optical scanning the interferences due to broad bands and continuum are removed. Potassium and rubidium were determined in an oxygen-hydrogen flame while the strontium was determined in nitrous oxide and air (80-20) -acetylene flame mixture.

The rubidium in the base material and at the 0.02 level, was extracted with sodium tetraphenylboron (NaTPB) in a mixture of methylisobutyl ketone (MIBK) and cyclohexane (3:1) [36]. The addition of the cyclohexane prevents the formation of an emulsion during the equilibration without affecting the distribution coefficient. The rubidium is determined in the organic phase with appropriate standards prepared with NaTPB in MIBK and cyclohexane. A summary of the results is presented in table 14.

As a check of the accuracy of this method, the values for potassium, rubidium and strontium at the 0.02, 1 and 50 ppm level were compared with the technique of isotope

Table 14. Summary of values for simulated lunar glass.

Sample	Element	Sample	Replicates	Conc., µg/g	Std. dev.	Rel. std. dev., %
Base Material	K	8	6	36.9	0.92	2.5
	Rb	8	2	<.3	-	-
	Sr	8	2	39.1	.66	1.7
0.02 ppm	K	7	2	29.1	.55	1.9
	Rb	7	2	<.3	-	-
	Sr	7	2	43.1	.63	1.5
1	K	8	2	29.9	.32	1.1
	Rb	8	2	0.82	.03	3.7
	Sr	8	2	47.2	.65	1.4
50	K	6	2	64.5	.83	1.3
	Rb	6	4	33.2	.41	1.2
	Sr	6	4	80.8	.70	0.86

Table 15. Comparison of flame emission and isotope dilution–mass spectrometry values in simulated lunar glass.

Sample	Element	Values reported µg/g		
		Flame emission	Mass spec.	Difference
0.02 ppm	K	29.1	29.2	-0.1
	Rb	.3	0.10	+ .2
	Sr	43.1	43.2	- .1
1	K	29.9	29.7	+ .2
	Rb	0.82	0.855	- .035
	Si	47.2	46.1	+1.1
50	K	64.5	63.4	+1.1
	Rb	33.2	31.4	+1.8
	Si	80.8	78.6	+2.2

dilution–mass spectrometry reported by the Mass Spectrometry Section. As shown in table 15, there is good agreement between the two methods for these three compositions.

2. Determination of Aluminum by Flame Emission Spectrometry in an Iron Matrix

The determination of aluminum by flame emission spectrometry in an iron matrix has been described previously [37]. However, with an oxygen–hydrogen or oxygen–acetylene flame, the resonance lines of aluminum have very low sensitivity and suffer from many spectral interferences. To overcome these interferences and increase the sensitivity, aluminum was extracted by early workers into an organic solvent which increased the sensitivity to 0.5 u g of aluminum per ml.

Slavin [38] has reported a detection limit of 0.1 µg/ml for aluminum by atomic absorption with a nitrous oxide–acetylene flame. By flame emission spectrometry using a fuel–rich nitrous oxide–acetylene flame, the sensitivity of aluminum in aqueous media was 0.1 µg/ml. The strong C_2, CH and CN bands, however, made the analysis difficult by the normal flame emission mode. With the repetitive optical scan [35], the interferences from the C_2, CH, and CN bands were eliminated and the sensitivity of aluminum was increased to 0.01 μg/ml.

A comparison of the precision of the two methods (emission vs absorption) was made using a ferrosilicon sample (NBS-SRM-59a). To correct for any matrix effect due to the iron, aluminum standards were prepared to contain the same iron concentration as the samples. The iron used in the preparation of the standards was high purity Mossbauer iron. The values obtained by absorption and emission on four replicates of five samples were 0.350 and 0.342 with standard deviation of .006 and .001 respectively. The improved precision by flame emission with the optical scan led to the use of this method for the determination of aluminum in several types of ferrous alloys.

Table 16 gives a summary of analyses by flame emission and atomic absorption spectrometry for the past year.

L. Service Analyses (T. C. Rains and T. A. Rush)

A large variety of samples submitted by other Divisions of the Bureau, as well as other Government agencies, were determined for various elements. These included sea water, high-purity barium metal, cell residue from an electrolytic process, ferrous filings, high-purity potassium chloride, zeolite, resin beads, calcium fluoride, and "polymer" water.

Lithium was determined in a series of sea water samples for the Naval Ordnance Laboratory by flame emission using the optical scan. The lithium concentration varied from 0.166 μg/ml for the unexposed sea water to 0.250 μg/ml for the sample in the test site. The relative standard deviation of the method was 1 percent.

As a service to a Division within the Bureau, sodium was determined in "polymer" water. The major difficulty is the analysis of a sample of less than 10 μl and the elimination of sodium contamination. To overcome these

Table 16. Summary of NBS-SRM analyses by flame emission and atomic absorption spectrometry.

NBS-SRM		Element	No. of detms	Conc., %	Std. dev.	Rel. std. dev., %
59a	Ferrosilicon	Al^a	20	0.342	0.001	0.3
		Ca^a	48	.0393	.0011	3.0
		Cu^b	38	.0519	.0007	1.4
125b	High silicon steel	Al^a	3	.328	.0020	0.6
1208	High Temp. alloy	Ca^a	8	.00136	.00016	9
1134	High silicon steel	Al^a	3	.332	.0036	1.1
1140	Ductile iron	Al^a	13	.0081	.00036	4.0
1141	Ductile iron	Al^a	8	.0054	.00043	8.0
1142	Ductile iron	Al^a	3	.0848	.0008	1.0
				µg/g		
1263	Spec. steel	Mg^b	4	5.3±0.5		
		Ca^a	2	3 ±1		
		Ag^b	2	30 ±1		
		Zn^b	6	13 ±2		
1264	Spec. steel	Ng^b	4	8.3±0.2		
		Ca^a	4	3. ±1		
		Ag^b	3	65 ±2		
		Zn^b	4	33 ±0.7		
	d-glucose	Mg^b	2	<0.5		
		Ca^a	2	1 ±0.5		

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[a] Flame emission spectrometry

[b] Atomic absorption spectrometry

problems small plastic tubes were mounted in plastic support caps and cleaned. The tubes were individually tested and placed in a desiccator. After the addition of 25 µl of ultra pure water to each tube, 2 to 5 µl of the sample of polymer water was added. The total sodium content was then determined by flame emission using the repetitive optical scan.

Magnesium, calcium and strontium were determined in a sample of barium metal. The protective oil coating was removed and the metal dissolved in dilute acid. The calcium and strontium were determined by flame emission using the optical scan in an oxygen–hydrogen flame and found to be 0.06 and 0.68 percent, respectively. The concentration of magnesium was determined by atomic absorption and was 0.0045 percent. By these methods barium did not interfere in the determination of magnesium, calcium, or strontium.

A number of the other analyses performed included rubidium in a cell residue, nickel, manganese, and chromium in ferrous filings, sodium and rubidium in zeolite, calcium and magnesium in cell buffer solutions, trace as well as the major components in laser glass.

4. ANALYTICAL COORDINATION CHEMISTRY

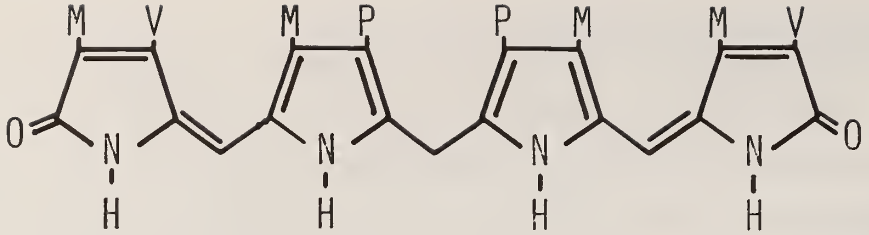
The various programs of the Section (ACCS) in both instrumental and classical analytical methods are interrelated with problems of solution complexation and coordination chemistry. This portion of the report represents some of the background work reported in the other competences. In evaluating spectrophotometric methods for the characterization of bilirubin, an investigation of its complexation with metal ions was undertaken. Similarly the spectrophotometric method for the determination of vanadium [39] led to the evaluation of the equilibrium constants and the kinetics of the extraction of vanadium and iron with β-isopropyltropolone.

A. Bilirubin Complexation (R. A. Velapoldi)

In Clinical Chemistry, the analysis of bilirubin (BR), although not definitive in itself, aids greatly in determining the various causes of jaundice. In cases of severe jaundice of the newborn, however, the analysis plays a major role in establishing the necessity for total blood transfusion. Thus research in the analytical procedures and the characterization and development of standards for BR has been extremely important in past years [40].

One of the major analytical problems has been the preparation of stable standard solutions for calibrating spectrophotometric measurements. Bilirubin is unstable in solution with reported rates of degradation varying from 1 percent per day in chloroform to 40 percent per day in basic solution.

Bilirubin is produced by the metabolic breakdown of hemoglobin [41]. It is a linear tetrapyrrole consisting of two conjugated dipyrrole segments joined by a methylene bridge. Several tautomeric forms exist; two of which are the bis-lactam (I) and bis-lactim (II) structures [42]. Recent reports [43] suggest the existence of a hydrogen bound mono-lactim structure (III) which contains a nucleophilic

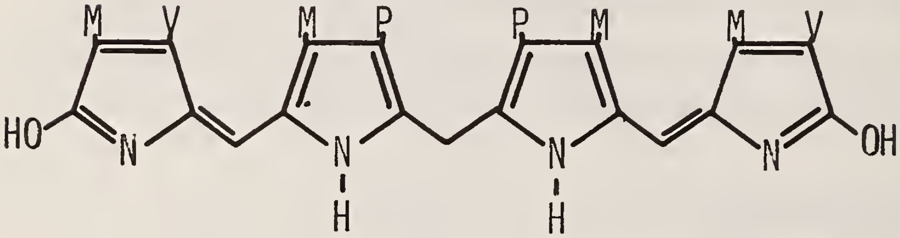


I. bis-Lactam

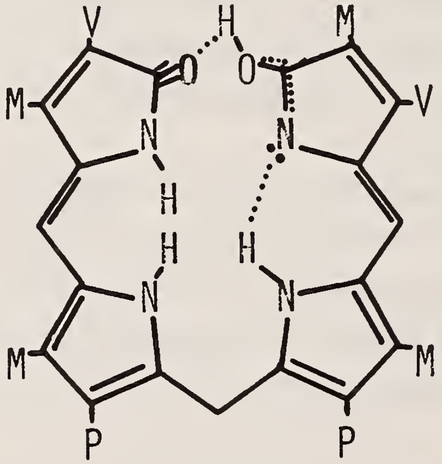
M = $-CH_3$

V = $-CH{=}CH_2$

P = $-CH_2-CH_2-COOH$



II. bis-Lactim



III. mono-Lactam - mono-Lactim
Hydrogen Bound Bilirubin

nitrogen and circular structure, both conducive to metal complex formation analogous to the porphyrin moiety. In addition, two brief papers [44] report the formation of a relatively stable zinc-bilirubin complex in ethanolic solution. With this information, a program was initiated to extend the study of metal-BR complex formations and to determine the feasibility of stabilizing bilirubin solutions by complexation with metals.

1. Solvent Studies

Bilirubin ($\varepsilon = 60{,}100 \pm 800$ liters cm^{-1} mole^{-1}) was dissolved in various solvents (chloroform; absolute ethanol: chloroform, 1:1; methanol: chloroform, 1:1; tetrahydrofuran; and dimethylsulfoxide). Ascorbic acid was also added to some solutions to determine its effect as an oxidation inhibitor. An inert atmosphere of nitrogen, further dried and purified by passage through a sodium-potassium alloy, was used in conjunction with a nitrogen manifold and specially constructed Schlenk-type spectrophotometric cells to insure air exclusion in specified solutions. Solvents used in these cases were distilled under nitrogen and were considered to be air and oxygen free. The best solvent system found for stability and complex formation was the 1:1 v/v ethanol: chloroform (see Stability Section).

2. Metal Complex Formation

Metal acetates, chlorides, or sulfates were dissolved in ethanol, added to BR solutions and spectral changes were observed with time. The following is an example of the method used: 0.05 cc of $2.42 \times 10^{-3}\underline{M}$ samarium(III) acetate in ethanol was added to and mixed with 1.3 cc of a $4.6 \times 10^{-6}\underline{M}$ BR solution in ethanol: chloroform resulting in a 4:1 molar ratio of Sm(III) to BR. Spectra were recorded at 25.0°C versus elapsed time on a recording spectrophotometer equipped with constant temperature cell blocks. A typical series of spectra obtained after

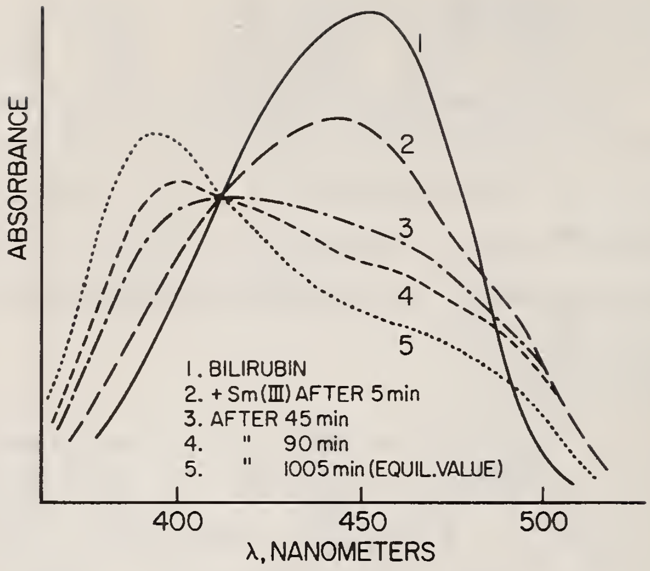


Figure 45. Addition of Samarium(III) to bilirubin solution results in blue spectral shift.

given elapsed times is presented in figure 45. Spectral shifts obtained by addition of various metal ions to BR are summarized in table 17.

Table 17. Metal ion addition to BR solutions results in various spectral shifts.

	Wavelength, nm			
Metal[a]	Peak 1	Stability	Peak 2	Stability
Zn(II)	476	No		
Cu(II)	420	No	352	No
Co(II)	423, 470(B)	No		
Ni(II)	476	No		
Fe(II)	424	No	463	Yes
Fe(III)	450(B)	No		
Sm(III)	395	Yes		
Nd(III)	391	Intermediate		
La(III)	425(B)	No		
Ca(II)	464, 495(S)			
Na(I)	no shift			

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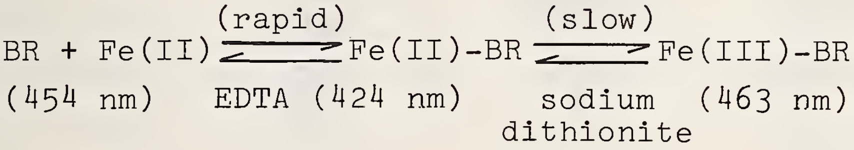
[a] [M]/[BR] = 4, (B) = broad, (S) = shoulder.

As can be seen, the addition of two metals, Fe(II) and Sm(III), resulted in stable complex formation. Spectral shifts were both hypsochromic (violet shift) and bathochromic (red shift) suggesting complex formation and not simply salt formation or interaction with the propionic acid functional groups. After reaching equilibrium values the addition of strong coordinating ligands (ethylenediaminetetraacetic acid or 1,2-diaminocyclohexanetetraacetic acid) to the Sm(III)- and Fe(II)-BR complexes resulted in a shift back to the free BR spectrum (454 nm), proving metal complexation with BR and not metal complexation with oxidized BR (which yields biliverdin, known to form metal complexes) [42, 43].

The addition of Fe(II) to BR results first in a rapid hypsochromic shift followed by a slower bathochromic shift. The addition of a reducing agent, such as sodium dithionite to the iron complex absorbing at 463 nm results in a hypsochromic shift to approximately 430 nm. This produced shift, observed in similar iron-porphyrin systems [45], is indicative of Fe(III) reduction to Fe(II). A proposed reaction scheme follows for the iron-BR system:



$$\underset{(454\ \text{nm})}{\text{BR} + \text{Fe(II)}} \underset{\text{EDTA}}{\overset{\text{(rapid)}}{\rightleftharpoons}} \underset{(424\ \text{nm})}{\text{Fe(II)-BR}} \underset{\substack{\text{sodium}\\ \text{dithionite}}}{\overset{\text{(slow)}}{\rightleftharpoons}} \underset{(463\ \text{nm})}{\text{Fe(III)-BR}}$$

The presence of an isosbestic point observed in the spectra during the oxidation step suggests the presence of Fe(II) and Fe(III)-BR complexes only. By using a curve resolver to separate Fe(II) and Fe(III) spectra and thus relative concentrations, plots of relative concentration versus time were obtained giving straight lines indicating second order kinetics for the oxidation step.

The most stable lanthanide complex is formed with Sm(III). Neodymium(III) addition results in complex formation (hypsochromic shift to 391 nm). It is approximately ten times less stable than the Sm(III) complex. Lanthanum(III) addition does not result in a complex formation. Stability results may be attributed to increased covalency in Sm(III) (larger charge to size ratio).

Addition of metals, such as Cu(II), Ni(II) or Co(II), causes rapid BR oxidation. These ions have strong tendencies to form square planar complexes and in this case introduce a strain at the BR methylene bridge [46], which contributes to the rapid oxidation as observed by Fog [47].

3. Metal Concentration Effect

The effect of changing the concentration of metal ions with respect to BR concentration is summarized in table 18. The best molar ratio for complex formation was determined to be 4:1, metal:BR. Less than this ratio, as in Cu(II) or Co(II), results in incomplete complex formation, although disappearance of the BR absorbance peak occurs slowly.

Table 18. Effect of metal ion concentration on complex formation.

Metal	[M]/[BR]	Peak 1 (nm)
Zn(II)	100	492
	4	476
Fe(II)	4	424
	1	445(B)
Co(II)	10	420, 475(B)
	4	422, 471(B)
	1	425, 468(B)
Cu(II)	100	418
	4	421
	1	425
	0.1	no observable shift
Sm(III)	10	395
	4	395

(B) = broad, solvent = 1:1 Ethanol:Chloroform.

4. Stability Studies

Data obtained from the investigation of the stabilities of BR under various experimental conditions is summarized in table 19. From these data it is evident that a 1:1 methanol: chloroform mixture is not a good solvent for BR. On the other hand a 1:1 chloroform: ethanol solvent system showed extremely low percent degradation in air or under N_2-0.051 percent per day versus 1 percent per day in chloroform. However, BR is not very soluble (∿5mg/100 ml) in this system and this factor is a drawback. It is not necessary to maintain solutions under nitrogen as the percent degradations for solutions 3 and 4 of table 19 were

Table 19. Stability studies of BR solutions under various experimental conditions.

No.	Solvent	Additions	Length of Test days	Percent Degradation per day[a]
(1)	$MeOH:CHCl_3$[b]	in air	5	particulate matter present in both tests.
(2)	$MeOH:CHCl_3$	Ascorbic Acid	40	0.625
(3)	$ETOH:CHCl_3$	in air	40	.051
(4)	$ETOH:CHCl_3$	under N_2	63	.053
(5)	$ETOH:CHCl_3$	Ascorbic Acid	40	.086
(6)	$ETOH:CHCl_3$	+ Sm(III)	63	.045
(7)	$ETOH:CHCl_3$	+ Nd(III)	56	.495
(8)	$ETOH:CHCl_3$	+ Fe(II)	60	.051
(9)	Solution(4)	in refrigerator ∿4 °C	46	.013
(10)	Solution(4)	at room temp. ∿25 °C in dark	10	.301
(11)	Solution(4)	at room temp. ∿25 °C in light	4	24.73

---

[a] As measured by absorbance decrease

[b] 1:1 volume/volume

approximately the same. The lower percent degradation per day for the Sm(III)-BR complex (0.045 vs 0.051 percent for EtOH:$CHCl_3$ alone) is probably not significant. On the other hand, the Nd(III)-BR complex degrades approximately eleven times as rapidly; exemplifying differences in the lanthanide-BR complexes stabilities.

A short study was made to determine the effect of temperature and light on BR in solution. This study was made using solutions 4, 9, 10 and 11 of table 19. Solution 4 was kept in the dark and under refrigeration for the entire test period and showed lowest percent degradation, 0.013 percent per day . Solution 9 was kept in the dark and under refrigeration, but was warmed to 25°C and spectra were taken every two or three days. This solution showed 0.051 percent degradation per day. Solution 10 was kept in the dark at room temperature (24-25°C) and showed the next highest degradation, 0.301 percent per day. Solution 11 was kept at room temperature and in light and showed an average percent degradation of 24.73 percent per day - definite evidence that increased temperature and especially light increase the rate of BR disappearance.

Figure 46 presents spectra of solution 11 over the 4 day test. The final spectrum with peaks at 377 nm, 700 nm and a shoulder at 665 nm is undoubtedly due to biliverdin [48].

Resolution of individual curves and plotting the area (relative concentrations) versus time results in figure 47. This is a typical zero order plot suggesting a reaction sequence similar to the following:

$$
\begin{array}{lll}
CHCl_3 & + \; h\nu \rightarrow \cdot CHCl_2 & + \; \cdot Cl \\
\cdot CHCl_2 & + \; BR \rightarrow \cdot BR & + \; CH_2Cl_2 \\
\cdot BR & + \; \cdot Cl \rightarrow HCl & + \; BVD
\end{array}
$$

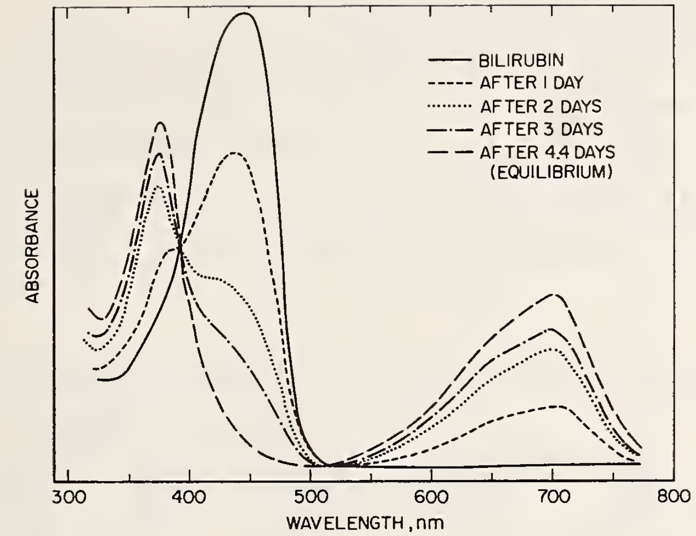


Figure 46. Spectra depicting photodecomposition of bilirubin to biliverdinoid pigment.

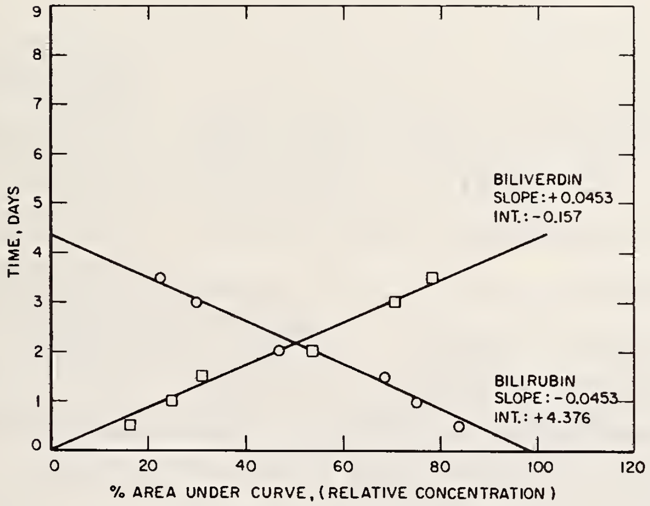


Figure 47. Formation of biliverdinoid pigment and disappearance of bilirubin during photodecomposition - relative concentrations as determined by curve resolver.

The same slopes for the disappearance of BR and BVD appear in figure 47 and the isosbestic point in figure 46 proves there are only two absorbing species in this reaction, BR and BVD.

5. Bilirubin Determinations

Quantitative BR analyses are performed usually by a diazotization reaction. The Malloy-Evelyn (ME) [49] and the Jendrassik-Grof-Nosslin (JGN) [50] methods were used for the determination of BR. Table 20 summarizes data obtained for both methods. The JGN method gives a better straight line calibration (no typical break in line at high concentrations) than the ME method, figure 48. However, the absorbance values are lower. Also plotted on figure 48 are the results of ME diazotization on the Sm-BR complex. The absorption peak in this case is shifted to 567 nm as opposed to 545 nm for usual diazotization. The interesting fact, however, is that increased absorbance readings are obtained indicating a higher molar absorptivity for the Sm-diazotized BR than the diazotized BR alone. The previously mentioned solubility problem coupled with the extra analytical step (addition of Sm(III) and equilibration) are deterrents to further developmental work on this system; however, metal addition after diazotization may be useful.

Table 20. Quantitative bilirubin analyses by diazotization.

Concentration mg/100 ml	Absorbance ME	Absorbance JGN	Std. dev. ME	Std. dev. JGN	Rel. std. dev. ME	Rel. std. dev. JGN
1	0.056	0.035	0.005	0.004	9.45	11.87
5	.203	.132	.006	.003	3.12	2.32
10	.434	.238	.018	.007	4.08	2.86
20	.715	.515	.010	.005	1.38	0.979

To increase solubility, the polar solvents dimethylsulfoxide, tetrahydrofuran and potassium cyanide in dimethylformamide were used. However, no metal-BR complexes formed, probably due to 'stronger' solvation of the metal ions.

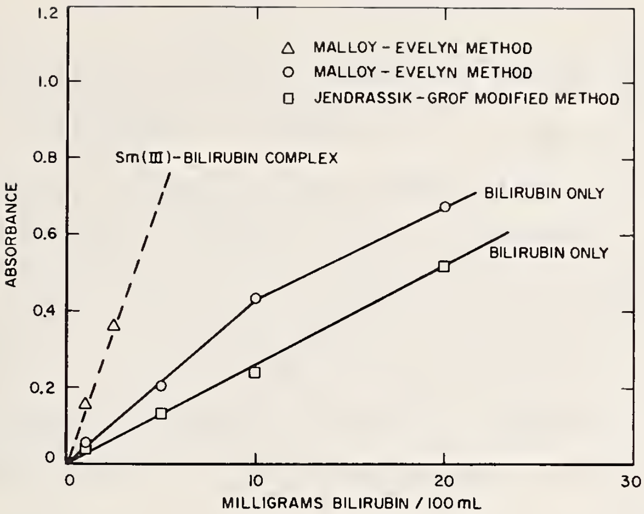


Figure 48. Standard calibration curves for diazotization reactions of bilirubin and Sm(III)-bilirubin complex.

6. Extraction

Attempts were made to extract a chloroform solution of BR by various concentrations of perchloric acid solutions of Fe(II), Zn(II) and Sm(III); however, no extraction occurred.

7. Complex Preparation

Several attempts were made to prepare and isolate for characterization the solid metal-BR complexes by: (a) addition of a hot solution of metal acetate in glacial acetic acid to a refluxing solution of BR in chloroform, followed by refluxing for varous times [51]; (b) use of tetrahydrofuran as solvent and proceed as in (a) under anhydrous conditions in an inert atmosphere; (c) reflux 50 mg BR and 200 mg metal acetate with and without sodium acetate in 50 ml ethanol for various times [52]; and (d) simple addition of scaled up (from spectrophotometric studies) amounts of the metal acetate in ethanol to BR in chloroform. All the above were unsuccessful.

8. Summary

The stability of BR in solution was increased substantially by use of a 1:1 ethanol: chloroform solvent system or by metal complexation with Fe(II) or Sm(III). Other metal ion or ascorbic acid addition and different solvent use resulted in oxidation or degradation of the BR. Diazotization of the Sm(III)-BR complex by the (ME) method results in higher absorbance values than the usual BR diazotizations; however, decreased solubility and the extra analytical step are deterrents to further studies along these lines.

Metal-BR complexes do not form in more ionic solvents. Attempts at isolation of the complexes were unsuccessful; however, interesting chemistry is involved and successful isolation followed by characterization would elucidate some of the problems now associated with the chemical and structural aspects of bile pigments.

B. Determination of Formation Constants of Iron(III) and Vanadium(V) with β-Isopropyltropolone (O. Menis, B. E. McClellan and D. S. Bright)

The role of adduct formation in the chelate extraction systems is of interest in methods used for analytical separations. This led to the study of the β-isopropyltropolone (HIPT) chelates of VO_2^+ and Fe^{+++} and to the comparison of the values of the various equilibrium constants calculated by graphical and least square methods. Dyrssen [53] studied the extraction characteristics of many metals with (HIPT). Dyrssen [53] as well as Singh and Dutt [54] have also determined the various stability constants of Fe^{+++} with HIPT using spectrophotometric methods. Hseu [55] also determined spectrophotometrically the pK_a of HIPT at several ionic strengths. Recently, Menis and Iyer [39] studied the extraction of VO_2^+ with HIPT for the separation and determination of vanadium in steel samples. The current investigation led to the establishment of the several extraction equilibrium constants of the adduct complex of VO_2 IPT.HIPT and new values for the $Fe(IPT)_3$ complex.

Equilibrium constants of ferric and vanadyl ions for the formation of the chelate with β-isopropyltropolone (HIPT) were determined by the extraction method. The values for these constants were derived by graphical and least squares computer calculations. The constants for the vanadyl system are reported for the first time. It is postulated that an adduct VO_2IPT:HIPT is formed and its formation constant, $\log \beta_N = K_1\ K'$, was determined as 13.8 and 13.6 (standard error of 0.8) by graphical and least square calculations, respectively. The partition constant, K_{DC}, was 2.5 and 2.6 ± 0.4 respectively. The values for the $Fe(IPT)_3$ complex were for $\log \beta_N$, 37.7 and 37.8 ± 0.1, respectively, while the $\log K_{DC}$ was 2 and 2.0 ± 0.1, respectively. It was also possible to calculate the overall stepwise constants, $\log \beta_1 = 13.0 \pm 0.2$ and by $\beta_2 = 24.8 \pm 0.1$ by the least squares method, the uncertainties indicated are standard errors of the computed values. The other experimental parameters studied were the effect of solvent and ionic strength.

Details of this study are presented in a manuscript submitted for publication in Analytical Chemistry [56].

C. Kinetics and Mechanism of Extraction of Iron(III) with β-Isopropyltropolone

Honaker and Freiser [57] and McClellan and Freiser [58] pointed out that, in favorable cases, solvent extraction can be used to study the rates of rapid chelation reactions. Many workers [53, 54, 59] have studied the extraction characteristics of metal ions with β-isopropyltropolone (HIPT).

Dyrssen [53] indicated that the extraction of Fe(III) with HIPT occurs very slowly from acid solution, and postulated that some step in the formation of the extractable complex, $Fe(IPT)_3$, is slow. The purpose of this study was to apply the extraction technique for studying the kinetics of reaction to the Fe(III)-HIPT system. It was hoped that an analysis of the kinetic data would lead to the elucidation of the mechanism and rate determining step in the extraction process. Iyer and Menis [39] found VO_2^+ to extract relatively rapidly (2 hours) into $CHCl_3$ compared to the slow (1-4 days) extraction for Fe(III) reported by Dyrssen [53]. Both of these metals extract from acid (1M) solution. Hopefully, a detailed study of the kinetics of extraction of the system would lead to a method for the separation of Fe(III) and VO_2^+ based on the large difference in the extraction rates.

The kinetics and mechanism of the reaction between Fe(III) and β-isopropyltropolone (HIPT) were studied by the extraction technique. The reaction was found to be first order in Fe(III) and in HIPT, indicating the addition of the first ligand to the Fe(III) to be the rate-controlling step in the reaction. A first-order dependence on $NaClO_4$ was observed. No dependence on $[H^+]$ was observed in the rate under the acid (1M) conditions of the extraction. The rate constant for the reaction was 1.5×10^{10} M^{-1}. Addition of ethanol prior to extraction greatly enhances the reaction rate.

The full description of this study will be found in the manuscript submitted for publication in Analytical Chemistry [60].

5. GASES IN METALS

The importance of the interstitial elements, oxygen and nitrogen, insofar as their deleterious effects on the mechanical and physical properties of metals and alloys has been discussed in Technical Note 504 [39]. The problem of analysis for these elements in metals has, from 1925 [61] until the present, been a continuing interest of the National Bureau of Standards. The current problems of analysis were investigated by using several approaches which will be discussed in the following sections of this report.

A. The Inert-gas Fusion Apparatus for the Simultaneous Determination of Oxygen and Nitrogen in Metals (J. T. Sterling)

It was shown in the past, that complete recovery of nitrogen in ferrous metals is possible provided the proper operating conditions are available. The conditions necessary for this complete recovery are a high operating furnace temperature (2000 °C) and the use for each analysis of a new or emptied reaction crucible.

The apparatus, as designed originally for these determinations, consisted of a small graphite crucible supported on a graphite pedestal whose cross section has been greatly reduced to minimize the heat loss due to conduction from the heated reaction crucible. Using this crucible arrangement it was demonstrated that nitrogen values for ferrous material agreed with those obtained by chemical solution methods [39].

The apparatus arrangement was selected to test a set of conditions and procedures and quantitatively extract the gases with a minimum of time and effort. Figure 49 is a schematic drawing of the apparatus that was constructed and used initially in this work. The glass interjoint was sealed with wax and during each determination this seal was melted. Therefore, changing the crucible between each analysis involved considerable work and time. Not only is this

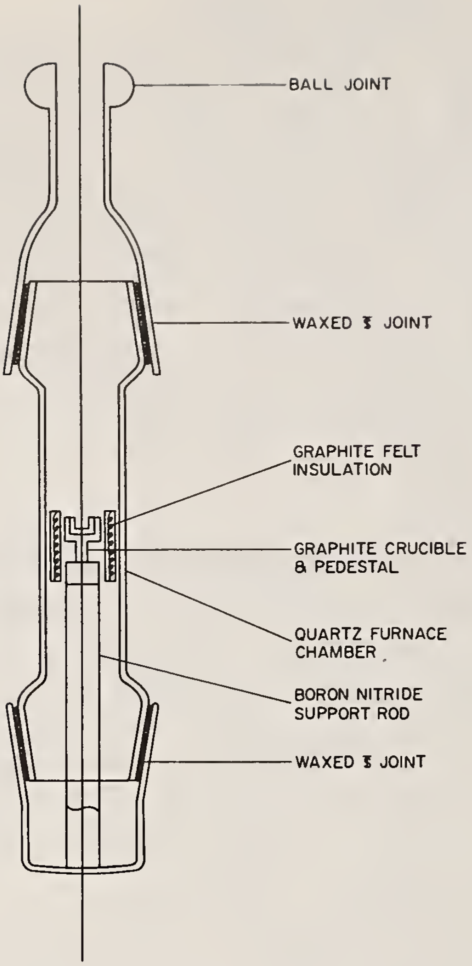


Figure 49. Crucible arrangement for inductive heating.

procedure time consuming, but it also allowed atmospheric gases to diffuse into the furnace section, thus increasing the apparatus blank and adding to the difficulties of outgassing of the furnace section. The furnace section of the apparatus was redesigned with the following modifications; see also figure 49.

(1) The diameter of the quartz reaction section of the furnace chamber was enlarged to provide room for additional insulation between the hot crucible and the water cooled furnace wall.

(2) A thin wall (0.003" thick) split radiation shield was installed between the crucible and the insulating material.

(3) The top of the reaction tube was simplified by eliminating standard taper adapter and relying on a single ball joint.

(4) A metal adapter between the furnace tube and the sample addition tube was designed and an O-ring groove was incorporated to eliminate any source of leakage.

(5) The closure for the bottom of the furnace section was newly designed and constructed of AISI 431 stainless steel. An air motor was adapted to close the bottom automatically when the electrical circuit was actuated. It was experimentally established that the removal and replacement of the reaction crucible with a sample can now be accomplished in less than 12 seconds.

(6) It is also planned to install several solenoid operated gas valves to reverse the gas flow in the furnace section. This would essentially eliminate that part of the blank due to gases absorbed from the atmosphere on the surface of the furnace reaction zone.

B. Evaluation of a Special Ingot Iron for Use as a Standard Reference Material for Oxygen Content (J. T. Sterling)

This special iron (NBS 1099) is the first of a series of five ferrous materials which will be investigated for use as a standard for oxygen in metal. This material is unique in that it is part of a larger program in which a single heat of metal will be processed to form the following:

(a) Rods 1-1/4 inches in diameter for use as optical emission and X-ray spectrometric standards.

(b) Rods 1/8 inch in diameter for application in electron microprobe analysis and spark source mass spectrometric analysis.

(c) Chip material for wet analytical analysis.

(d) Rods 1/4 inch in diameter for gases in metals.

It then will serve as a single source of standards covering the analytical methods of interest.

The material under (d) for gas analysis has been designated as SRM 1099.

Preliminary testing of this material has begun with the results shown in table 21. From a study of this table it is noted that one of the rods (A1T) from batch A has a significantly higher oxygen content. It was therefore decided to analyze each bar before the lot was certified. This work is in progress, and should be completed in two months.

Table 21. Study of homogeneity in SRM 1099.

Rod No.	Mean O_2[a]	Standard deviation	Relative standard deviation
	ppm	ppm	%
A3T	62.4	1.84	2.94
C4T	66.5	2.03	3.05
C3T	65.2	2.36	3.62
C4L	63.3	2.25	3.55
A1T[b]	131.4	19.68	14.97
C2L	66.8	0.91	1.36
A7T	59.0	1.45	2.46
A3T	61.4	1.61	2.62

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[a] Average of 3 determination.

[b] One of a series of rods which has a higher oxygen content.

6. THERMAL ANALYTICAL METHODS

A. Introduction

The need for materials that are suitable for use as temperature standards in differential thermal analysis methods has been discussed in earlier editions of the NBS Technical Notes 454 [17] and 504 [39] and the need for these standards continues.

The exigency for these standards has been shown by the attention paid to this problem in recent years by the Committee on Standardization of the International Confederation for Thermal Analysis. Currently the newly formed Subcommittee on Thermoanalytical Test Methods in Committee E-1 of the American Society for Testing and Materials has delegated to one of its major study groups the responsibility for the preparation and procurement of these standards. Progress in the standards programs of both groups as related to the participation by the National Bureau of Standards as well as the programs of this Section for issuing two new Standard Reference Materials will be discussed.

B. The Second International Cooperative Program on Standardization by the ICTA (J. T. Sterling and O. Menis)

There have been circulated to about forty laboratories throughout the world, nine samples of materials that have been proposed as temperature standards for differential thermal analysis. These nine samples included potassium nitrate and quartz which were provided by the National Bureau of Standards. The latter will be discussed separately in this report.

All of the materials, except strontium carbonate, were analyzed with instrument #1, to determine the temperatures of the solid→solid or solid→liquid transformation. This equipment, with the calibrated thermocouples inserted into a capillary tube has been modified to permit the recording of the sample temperature and the differential thermal curve by means of a variable span, adjustable zero two pen recorder as described in Technical Note 504. The strontium carbonate was analyzed using the modified apparatus (Instrument II) which is also described in NBS Technical Note 504.

The conditions used in these determinations were as follows for both instruments, except as noted:

Reference material - Alumina (Al_2O_3)
Weight reference material - approximately 0.1g
Furnace atmosphere - Dry nitrogen
Flow rate - 500 cc min^{-1}
Pressure - Atmospheric
Heating Rate - 5°C min^{-1}
Sample Weight - Approximately 0.1g
Sample Container - Instrument I - 4 mm dia. quartz tube
Instrument II - Platinum cup

The results of these studies in our laboratories are presented in table 22. Results from the cooperating laboratories are not available. However, a comparison of results obtained in this laboratory from the previous and current ICTA tests are presented in table 23. The second results show an overall improvement, in that large differences have been eliminated. In part they reflect the improvement in instrumentation. In all cases, as noted previously [39], the differential temperature indicates positive deviation on the heating cycle from the thermodynamic data obtained under adiabatic conditions. This can be attributed to the rate of heat transfer which is the controlling factor in DTA. The value listed in NBS Circular 500 for Ag_2SO_4 is quite different from the value obtained in this study. Similar differences have been reported by other cooperating laboratories.

Table 22. Observed transition temperatures for ICTA materials: A, departure point; B, intersept and D, peak.

Material	Heat °C						Cool °C					
	A		B		D		A		B		D	
	Mean	S.D.	Mean	S.D.	Mean	S.D	Mean	S.D.	Mean	S.D.	Mean	S.D.
$KClO_4$	299.8	0.500	300.8	0.500	301.5	0.577	290.0	0.000	290.0	0.816	291.5	0.577
K_2CrO_4	665.0	2.582	668.5	.577	670.8	.957	671.2	.957	671.2	.957	669.8	.957
Ag_2SO_4	426.5	0.577	427.5	.577	431.8	.500	407.0	5.477	407.0	5.477	413.5	3.873
K_2SO_4	583.5	1.291	586.0	.817	586.8	.500	587.2	0.500	587.2	0.500	587.0	0.000
Sn (metal)	233.0	0.000	233.0	.000	235.2	1.500	197.8	12.553	197.8	12.553	231[c]	-
In (metal)	157.8	.500	157.8	.500	158.2	0.500	155.2	1.500	155.2	1.500	149.0	1.155
KNO_3	129.0	.000	129.5	.577	130.8	.500	124.0	0.000	124.0	0.000	124.0	0.000
SiO_2 (quartz)	572.0[a]	1.225	574.4[a]	.548	575.4[a]	.548	578.8	.957	575.8	.500	574.5	.577
$SrCO_3$	925.0[b]	3.606	929.3[b]	1.527	936.0[b]	2.000	910.2	.957	908.8	.957	905.0	1.414

Note: The mean and standard deviation (S.D.) tabulated are based on four replicate runs, except as noted.

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[a] Based on 5 replicate runs.
[b] Based on 3 replicate runs.
[c] Based on 2 replicate runs.

Table 23. Results reported by NBS. First and second ICTA-DTA study.

Compound	Transition type	Circ. 500[a] °C	1st ICTA "B" °C	2nd ICTA "B" °C
KNO_3	s→s	127.7	129	129.5
$KClO_4$	s→s	299.5	300	300.8
Ag_2SO_4	s→s	412	426	427.5
SiO_2 (quartz)	s→s	573	570	574.4
K_2SO_4	s→s	583	577	586.0
K_2CrO_4	s→s	665	668	668.5
$BaCO_3$	s→s	810	791	-
$SrCO_3$	s→s	925	905	929.3
Sn	s→l	231.9	-	233.0
In	s→l	157	-	157.8

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[a] National Bureau of Standards Circular 500. Selected values of chemical thermodynamic properties (1952).

C. The ASTM Committee E-1 Subcommittee Round Robin on Thermal Analytical Substances (J. T. Sterling and O. Menis)

Under the chairmanship of O. Menis of NBS, the Standards Section of the ASTM Committee on Thermal Analytical Methods has conducted a round robin test on thermal analytical substances. Samples of three materials, intended to cover the lower temperature scale not studied by ICTA, were sent to sixteen laboratories which had indicated an interest in cooperating in such studies.

The results from nine laboratories cooperating in this study are shown graphically in figures 50a, 50b, 51, 52a and 52b. The means obtained for the departure "A", the intersection "B", and the peak "D" temperatures for the various transitions have been plotted against assigned laboratory numbers, (NBS No. 10). The NBS Circular 500 [62] values for the transitions occurring in these materials are given in table 24.

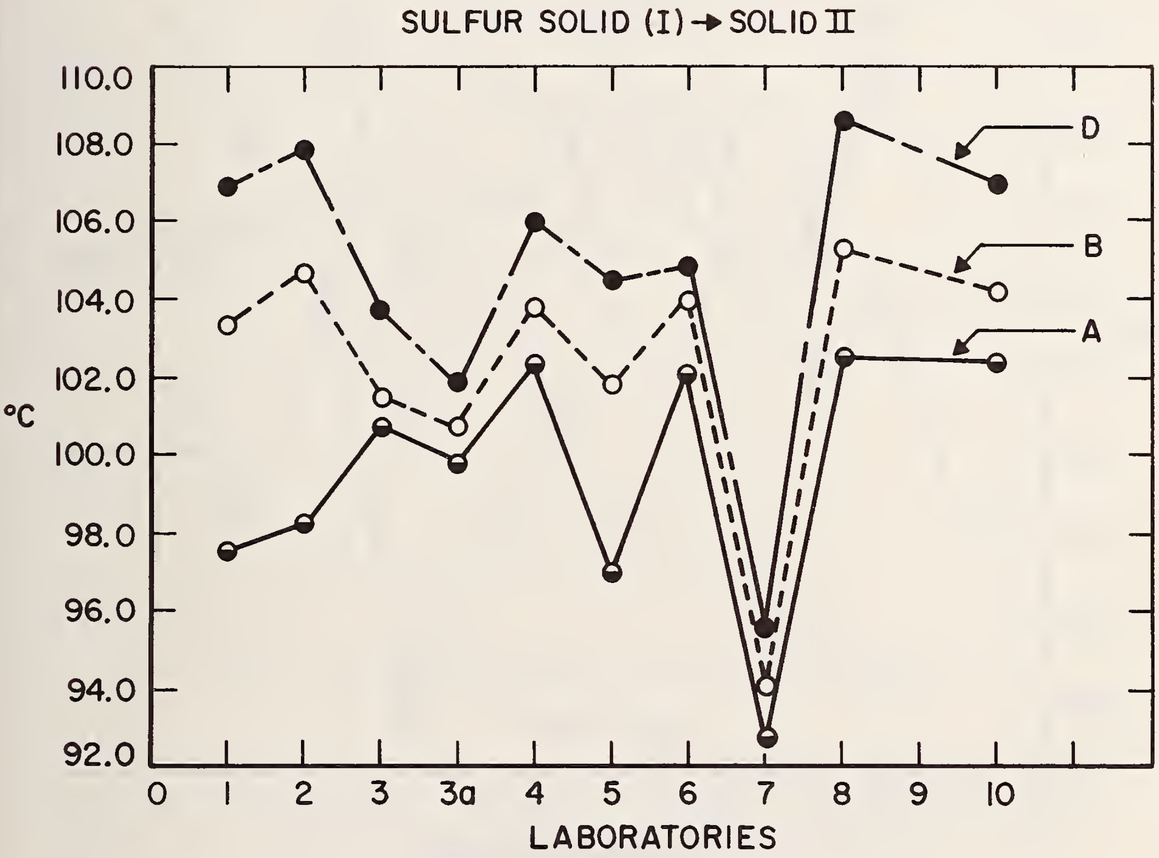


Figure 50a. Cooperative data for ASTM committee members (one member with two data points).

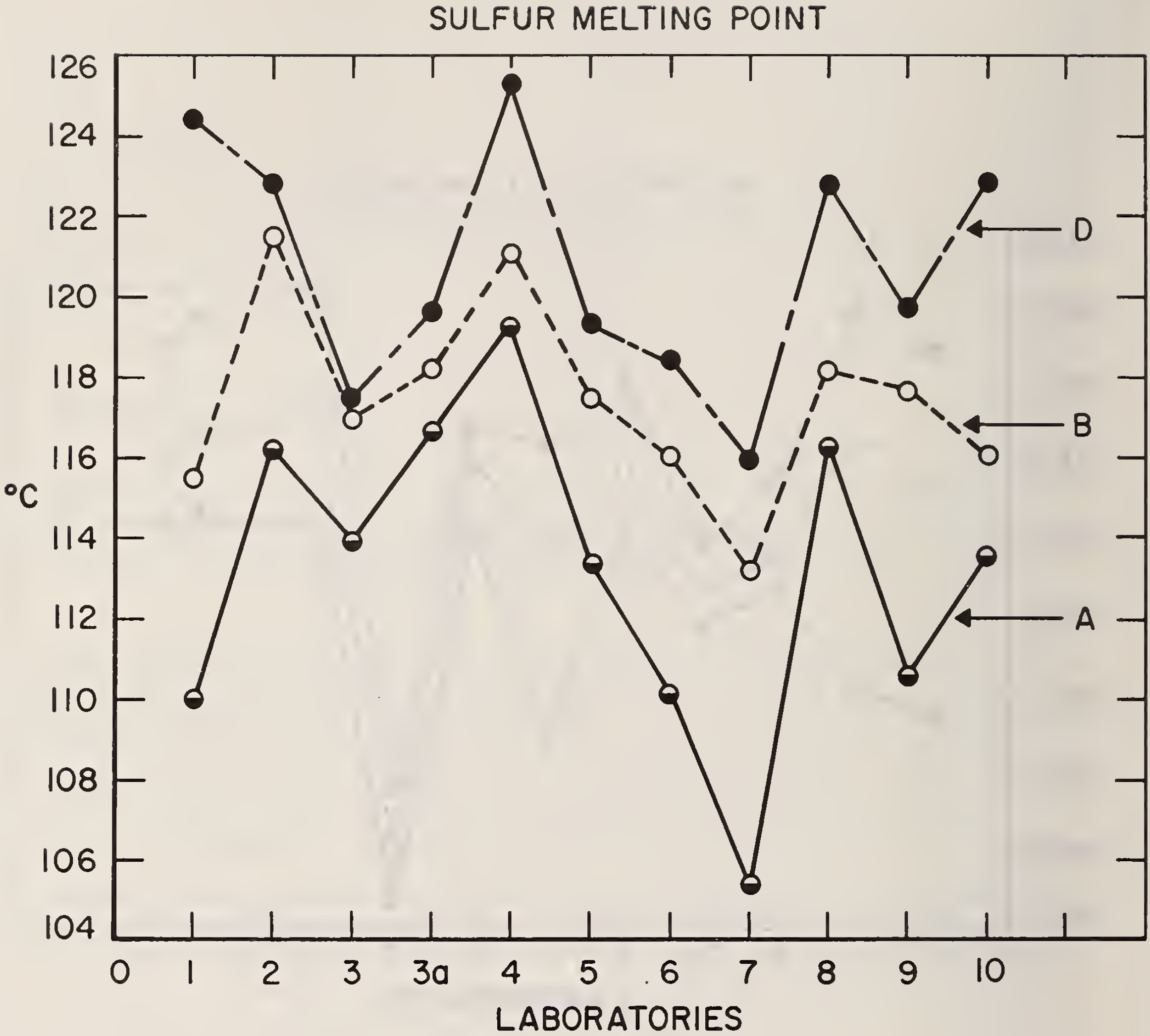


Figure 50b. Cooperative data for ASTM committee members (one member with two data points).

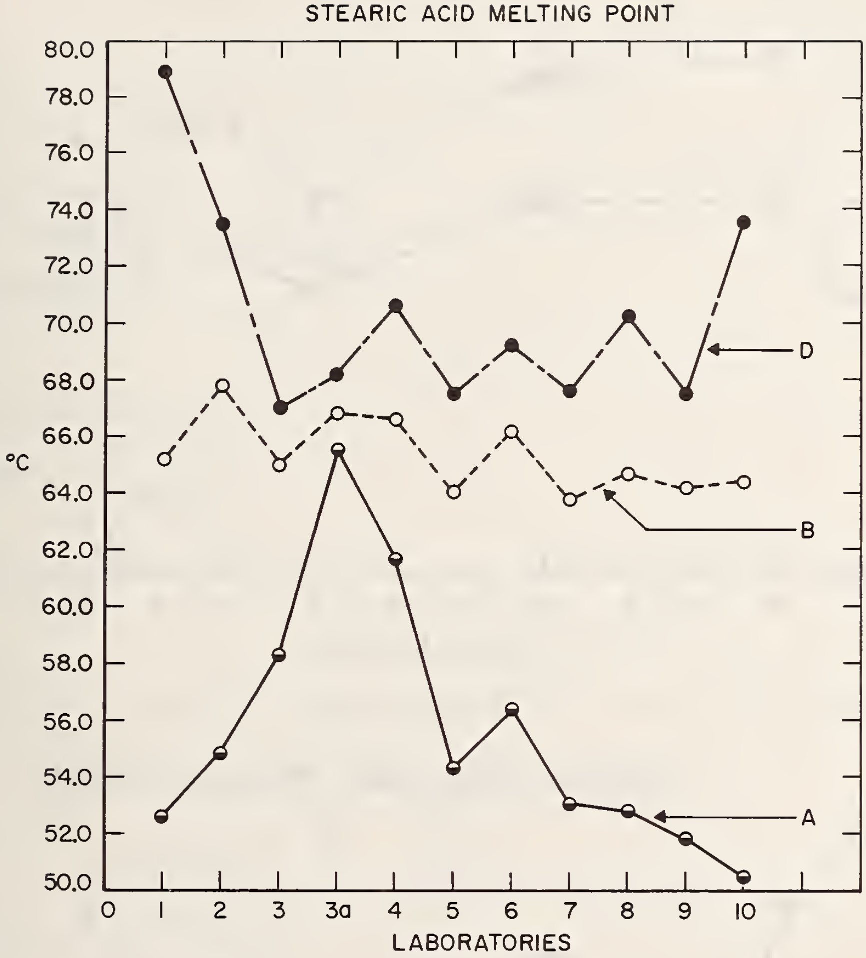
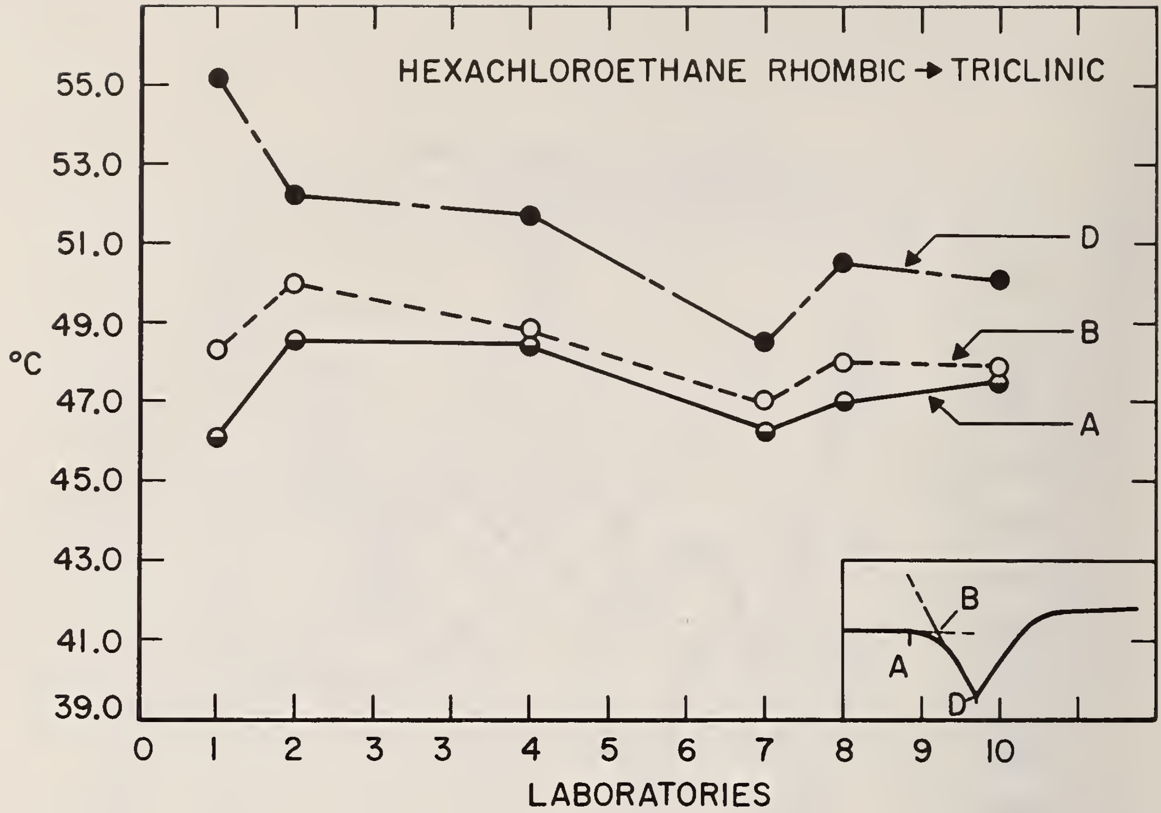
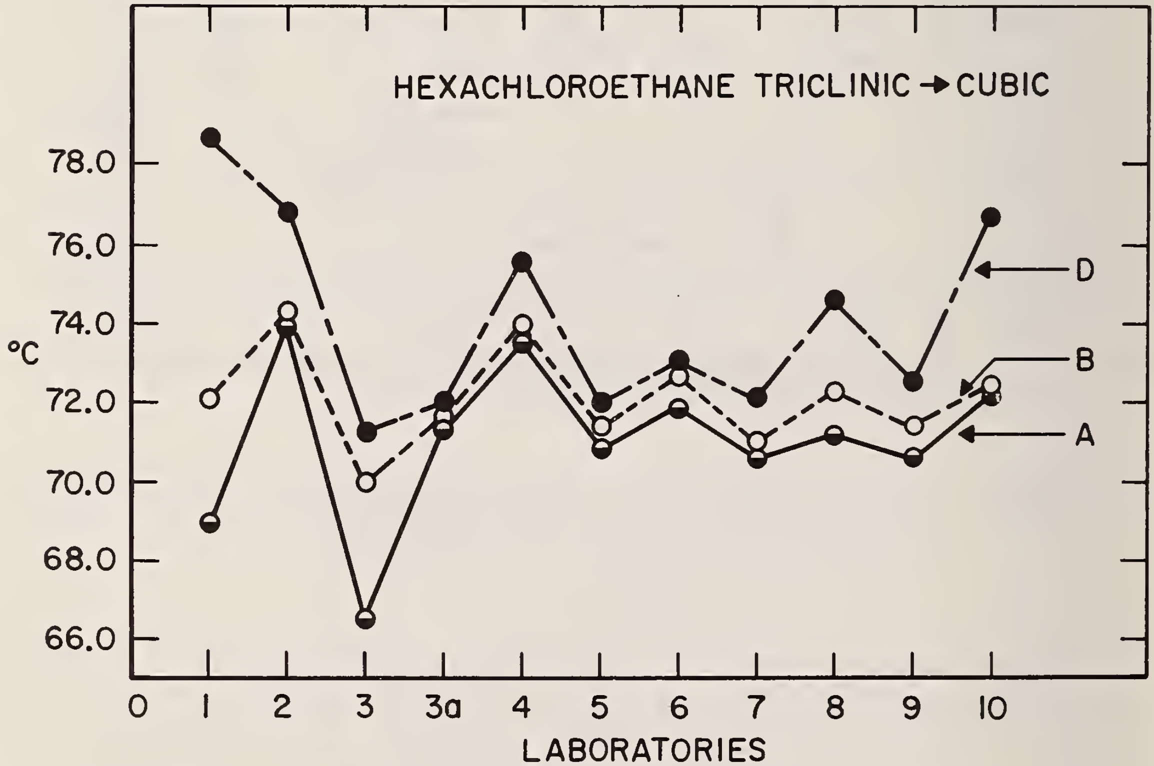


Figure 51. Cooperative data from ASTM committee members - for stearic acid melting point.





Figures 52a, 52b. Cooperative data from ASTM committee members.

Table 24. Transition temperatures—ASTM round robin materials. NBS Circular 500.

Material	Transition Type	Temperature °C
Sulfur	C_{II} Rhombic→C_{I} Monoclinic	95.4
Sulfur	C_{I} Monoclinic→Liquid	119.0
Hexachloroethane	C_{III}→C_{II}	(45.1)[a]
Hexachloroethane	C_{II}→C_{I}	71.2
Stearic Acid	C_{I}→Liquid	69.1

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[a] From reference only.

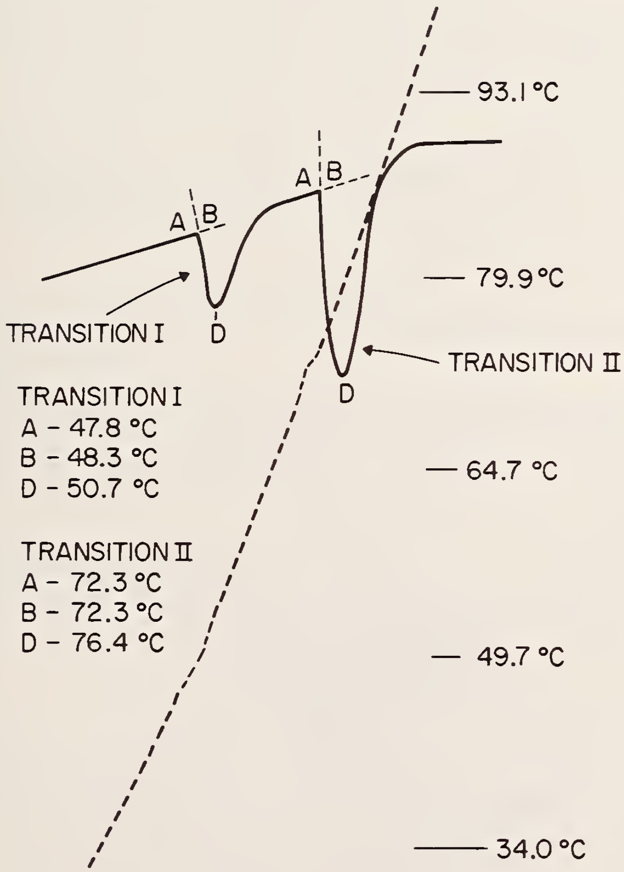

Figure 53. Thermal curve of hexachloroethane.

A study of the results of this investigation reveals that while some agreement between laboratories was attained, especially when the intersection "B" is used as the measure of transition temperature, there is still a wide disagreement between certain laboratories. Thus the need for Standard Reference Materials is again well supported by this latest study. Of the three compounds, hexachloroethane, shown in figure 53, especially because of its two transition temperatures at approximately 48° and 72°C, would be of great value for calibration purposes. An effort will be made to certify it as a Standard Reference Material.

D. Standard Reference Material 755, Quartz (SiO_2)

(J. T. Sterling)

The preparation and certification of Standard Reference Material 755, (Quartz) for use in checking the performance of differential thermal analysis equipment were completed during the last year.

This material is a natural crystalline quartz that has been ground, cleaned and sized to minus 100 mesh (0.149 mm) and plus 325 mesh (0.044 mm).

This material was analyzed at NBS and at one other laboratory.

The following conditions were used to determine the transition temperature of this reference standard.

Sample sizes - 3 to 300 mg

Sample container - Graphite or platinum cylindrical crucible, or alumina cylindrical sample block.

Heating rates - 1 to 10 degrees celsius per minute

Reference material - Alumina (Al_2O_3)

Atmosphere - Static dry air or nitrogen

Thermocouple - Located on the axis of the material with the sensing element or bead at or near the midpoint of the specimen.

The transition temperature obtained using the values recorded from three instruments in two laboratories is 575°C for the intersection point and 577°C for the peak point as normally observed in a differential thermal analysis curve [63].

An interesting experiment conducted in conjunction with the testing of this material involved the use of a single large crystal of the quartz. This crystal was from the same lot of material used for the Standard Reference Material. A cylindrically shaped test specimen 0.125 inch in length and 0.10 inch diameter was cut with a glass saw. A hole large enough to contain the DTA thermocouple was cut into the center of the quartz cylinder and along the long axis to a depth of approximately 1/16".

The differential thermal analytical curve was run, the transition temperatures being shown in table 25.

These data are in remarkable agreement with the reported thermodynamic value of 573°C [64].

Table 25. DTA transition temperatures for a solid quartz specimen.

	Heating Transition Temperature, °C	
	Intersection (B)	Peak (D)
Mean	574.1	574.1
Number of Det.	4	4
Standard Deviation	0.1	0.1
	Cooling Transition Temperature, °C	
	Intersection (B)	Peak (D)
Mean	573.1	572.9
Number of Det.	4	4
Standard Deviation	0.1	0.1

E. Standard Reference Material 756, Potassium Nitrate (KNO_3)

The preparation and certification of Standard Reference Material 756, (Potassium Nitrate) for use in checking the performance of differential thermal analysis equipment were also completed during the last year. This potassium nitrate was a specially prepared high purity material whose mesh size was approximately minus 50 to plus 100.

The following conditions were used to determine the transition temperature of this reference standard:

Sample Condition - Because the first heating of this material is not a reproducible measure of the transition temperature, the sample must be cycled through the transition temperature in situ before recording data. This precaution holds whenever the specimen is allowed to be at room temperature and exposed to the atmosphere for more than one hour.

Sample sizes - 3 to 300 mg

Sample container - Graphite, quartz or platinum cylindrical crucible or alumina cylindrical sample block.

Heating rates - 1 to 10 degrees centigrade per minute

Reference material - Alumina (Al_2O_3).

Atmosphere - Static dry air or nitrogen

Thermocouple - Located on the axis of the material with the sensing element or bead at or near midpoint of the specimen.

The average values based on three cooperating laboratories using different equipment are as follows:

Intersection - - - - - 130°C
Peak - - - - - - - - - 131°C

F. Thermal Gravimetric Analysis of Bilirubin (J. T. Sterling)

In conjunction with the certification of SRM's for clinical chemistry, samples of bilirubin and mandelic acid were analyzed by the thermal gravimetric method. The heating rate for both materials was 50°C per minute. Samples were run in dry air and in dry nitrogen.

Representative curves obtained under these conditions are shown in figures 54, 55, 56 and 57. For the bilirubin samples, a self-heating effect, probably due to oxidation of the carbonaceous material, takes place just below 300°C when the samples are heated in air. No similar effect was observed for bilirubin when it was heated in dry nitrogen. For the mandelic acid sample, the self-heating effect is not observed when the samples are heated either in dry air or nitrogen. From a study of these weight-loss curves along with other chemical data observed, it is possible to obtain valuable insights as to the best sources of materials which can be used as standard materials. It is postulated that samples having a higher and sharper decomposition temperature may be purer.

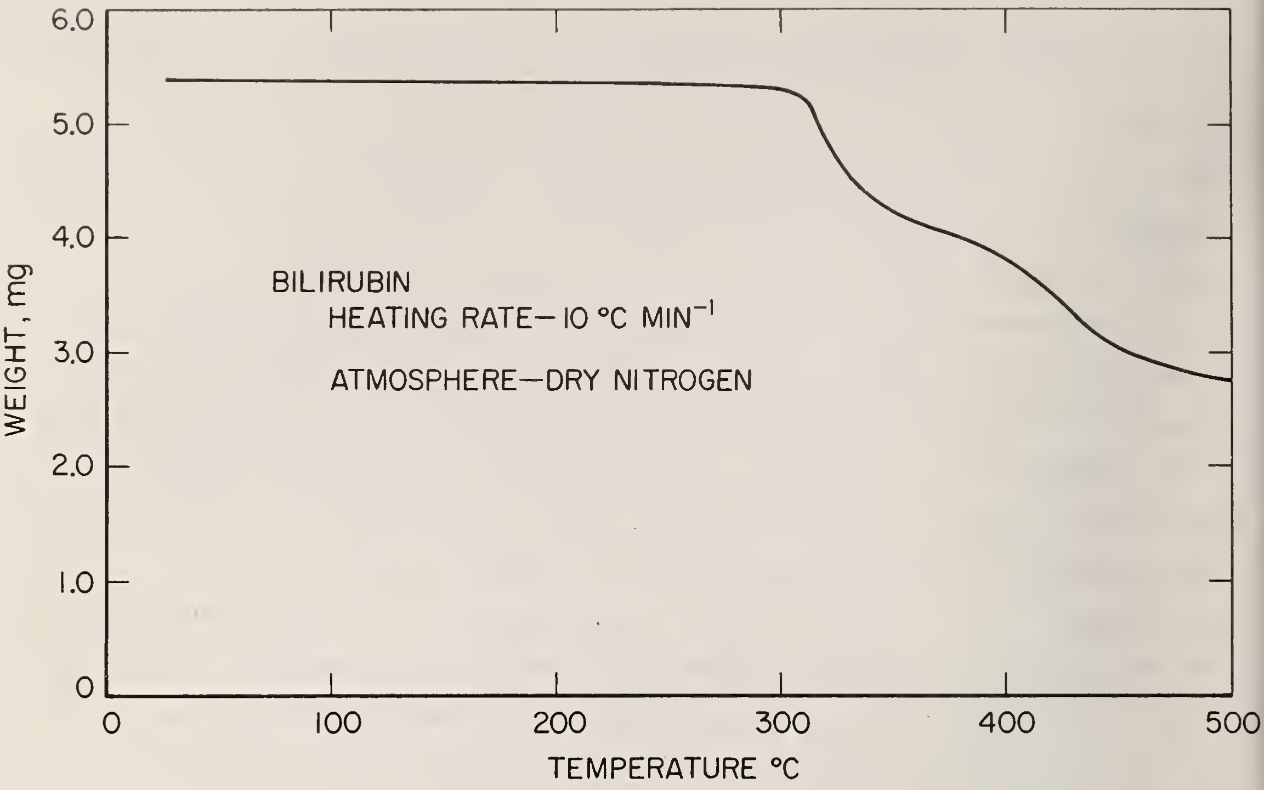

Figure 54. Thermogravimetric curve of bilirubin in a dry nitrogen atmosphere.

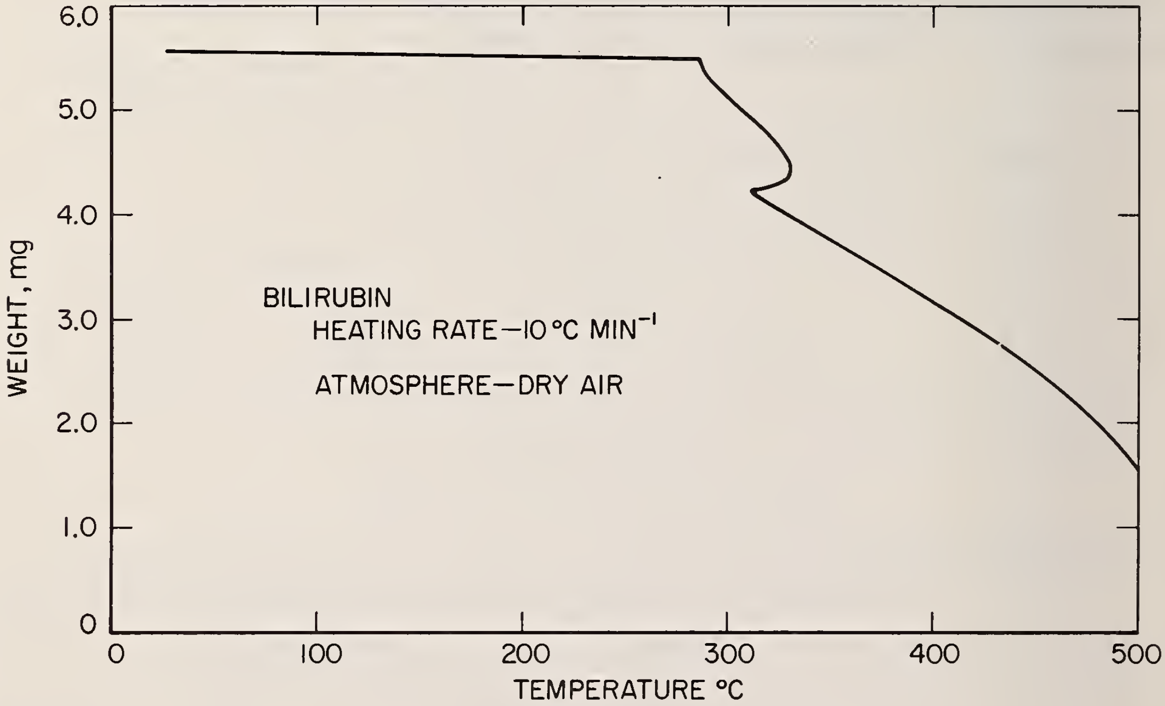

Figure 55. Thermogravimetric curve of bilirubin in a dry air atmosphere.

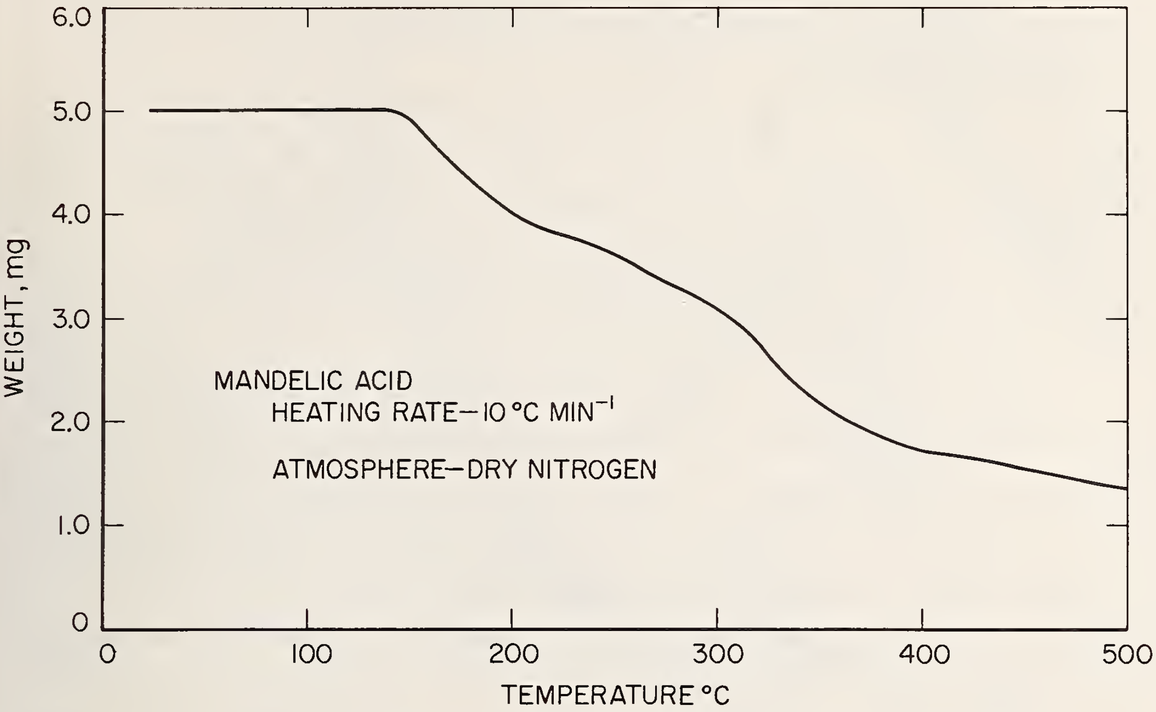

Figure 56. Thermogravimetric curve of mandelic acid in a dry nitrogen atmosphere.

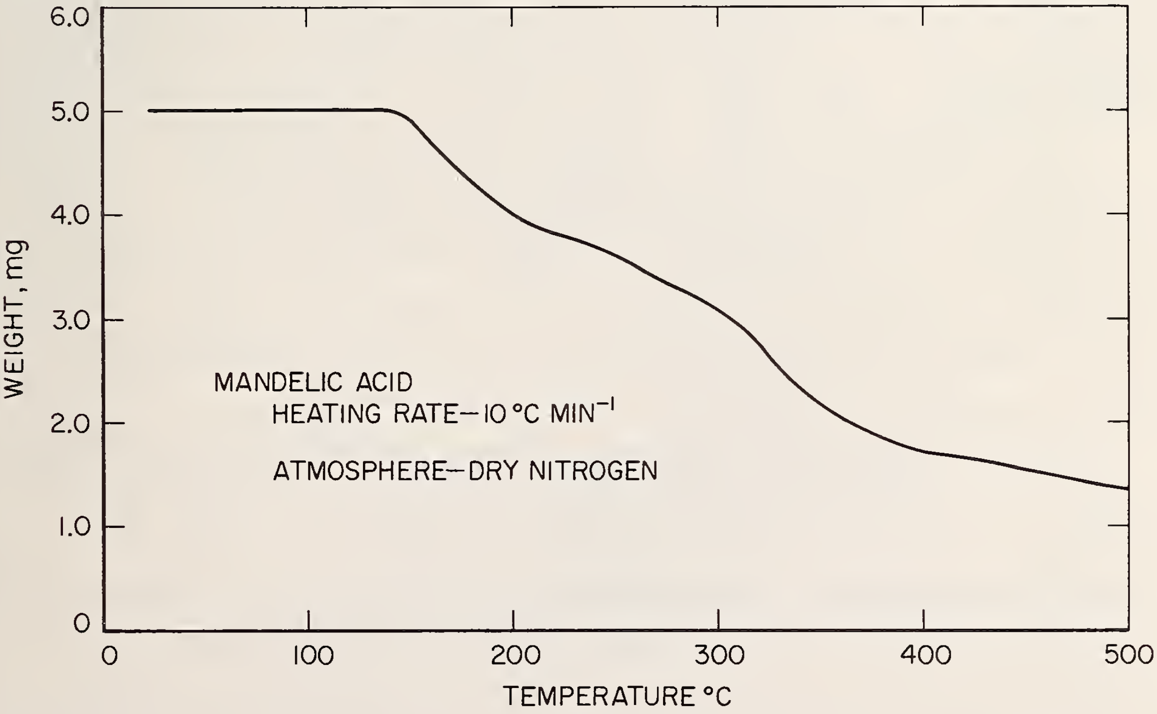

Figure 57. Thermogravimetric curve of mandelic acid in a dry air atmosphere.

7. ANALYSIS OF STANDARD REFERENCE MATERIALS

A. Introduction (J. I. Shultz)

At least 50 percent of the activities of the Section, during the last year, involved the analysis or characterization of a wide variety of materials for the Office of Standard Reference Materials. These activities require considerable versatility and experience on the part of the staff members.

Atomic absorption and flame emission spectroscopic, gravimetric, titrimetric, spectrophotometric, combustion-gravimetric and thermal conductivity techniques were effectively employed to cover the broad scope of concentrations from the parts-per-million level to at least 50 percent. As requirements for lower detection limits become increasingly more demanding, atomic absorption, flame emission, spectrophotometric and thermal conductivity techniques occupy positions of greater importance. However, for those materials containing elements in the ranges above 10 percent, as in ferroalloys, metallo-organics and clays, it becomes quite apparent that well established gravimetric and titrimetric procedures must be relied upon.

Investigations into possible new absorbance standards for use in calibrating spectrophotometric instruments concerned the development and evaluation of a number of solids and liquids. The details of the investigation and description of the proposed standards have already been given in a previous section.

The accompanying diagram, figure 55, more graphically illustrates the variety and types of standards that were analyzed either for provisional or final certification or for establishing homogeneity of the materials. Figure 56 identifies those elements that have been determined during the year by one or more of the competences within the Section in connection with the certification of Standard Reference Materials.

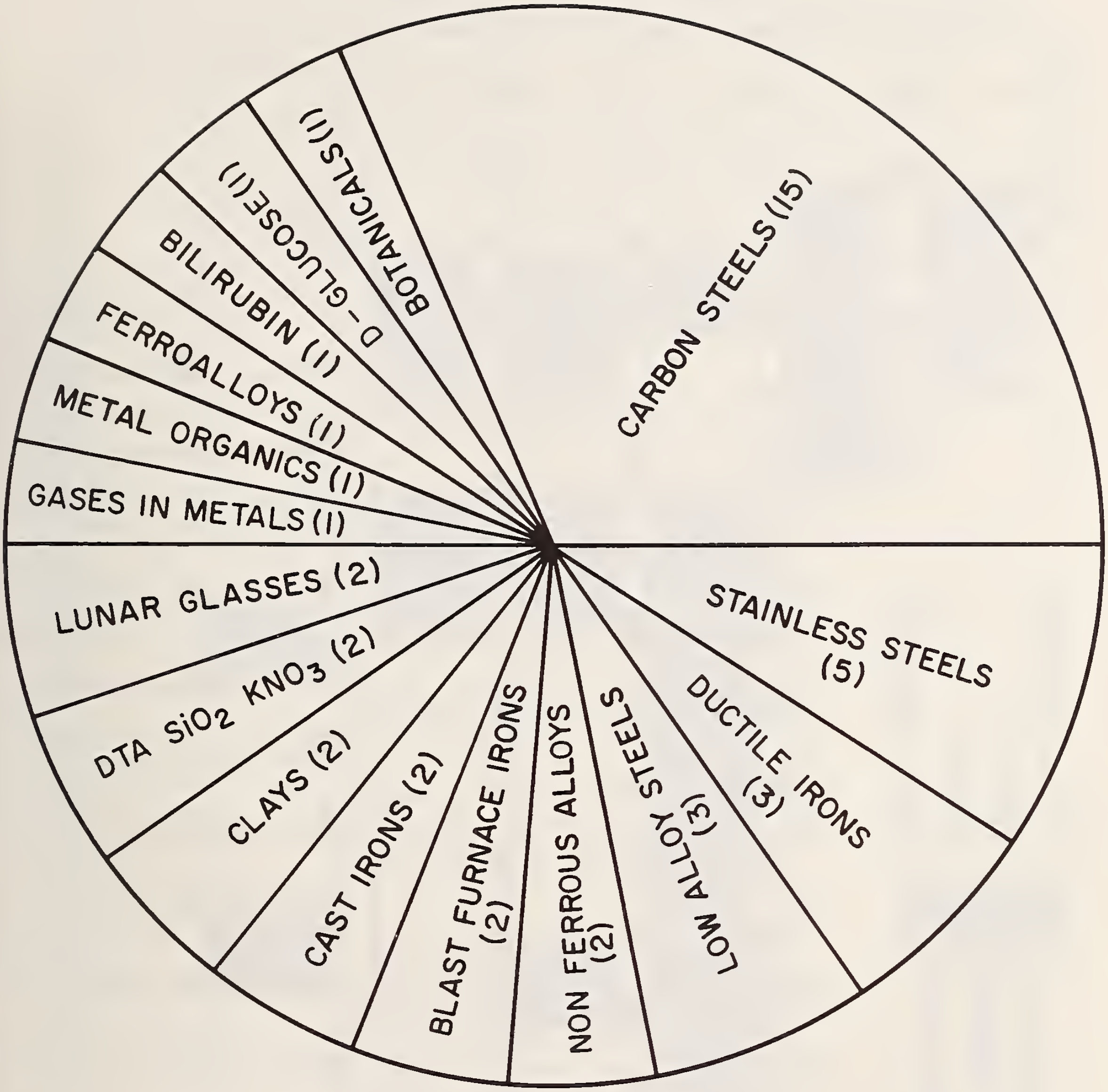

Figure 58. Types of Standard Reference Materials analyzed.

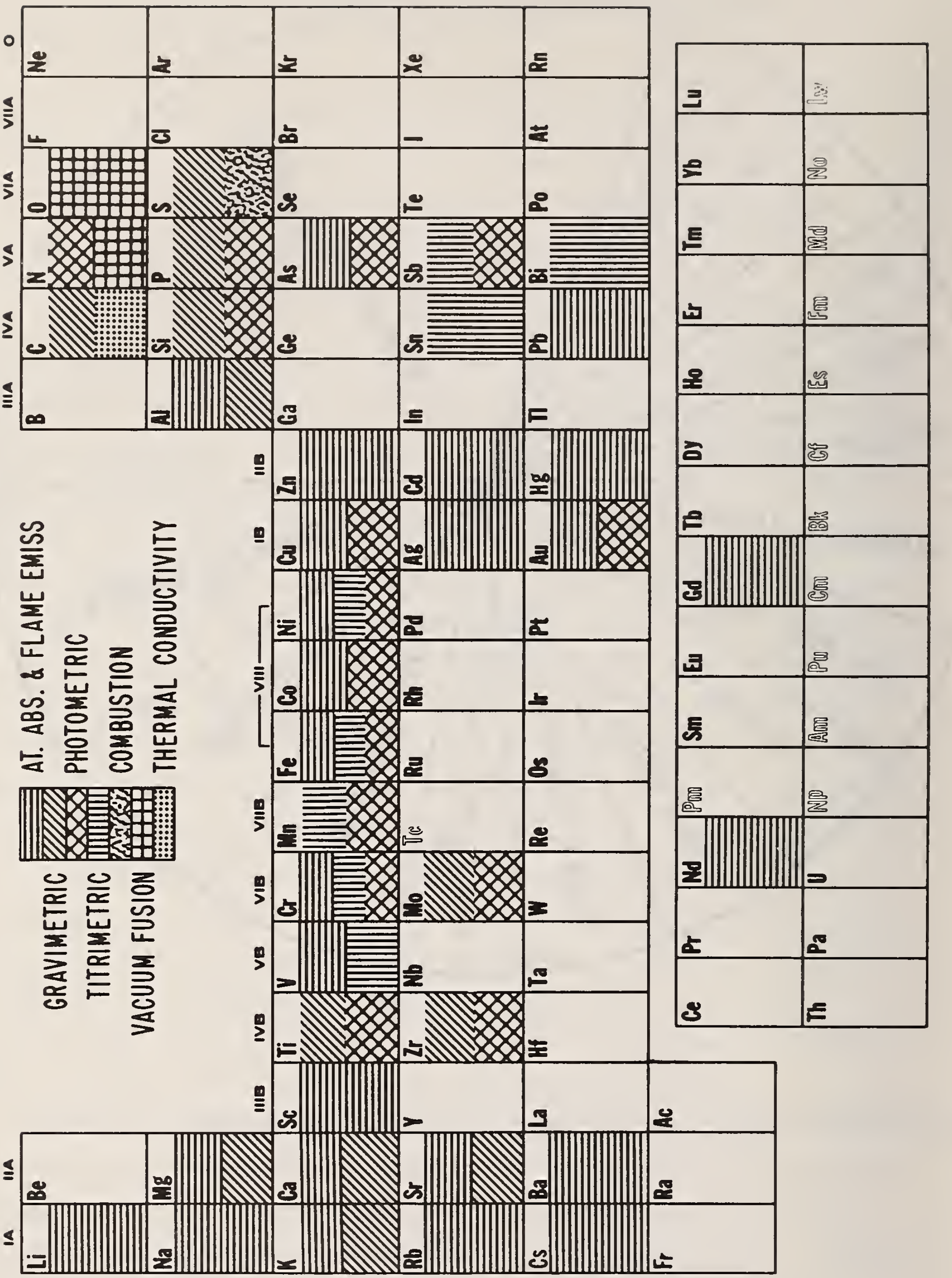

Figure 59. Elements determined according to competences by Coordination Chemistry Section.

B. Standard Reference Materials Information and Summary

The following information on those standards for which the Section was responsible for the overall direction and coordination of the technical measurements, provides background which may be of general interest.

1. Lead-bearing alloys-SRM 53e and 1132

Non-ferrous alloy 53e is a renewal standard of lead-base bearing metal in the form of finely-divided powder (170/325 mesh), while 1132 is a new standard of similar composition and is available in disc form for spectrographic use. These alloys are typical bearing materials widely used by the automotive and diesel industries. Composition control is becoming increasingly more important not only for the major elements, such as antimony (10%) and tin (6%), but for the minor elements, such as copper, arsenic, bismuth, iron and nickel. The data are presented in table 26.

Table 26. Certified lead bearing alloy-SRM 53e and 1132.

Element	No. Det'ms	Method	Average %
Antimony	4	Distillation-titration	10.22
	4	H_2SO_4-H_2SO_3-$KBrO_3$	10.24
Tin	8	KIO_3 titration	5.83
	4	Cupferron-SnO_2	5.86
Copper	8	Cupric bromide photometric	0.053
Bismuth	8	Thiourea photometric	.052
Arsenic	16	Arsenomolybdate photometric	.058
Iron	2	1,10-phenanthroline photometric	<.001
Nickel	8	Dimethylglyoxime photometric	.004

2. Carbon Steel (0.4C)-SRM 20g

This medium carbon steel is the most popular of the carbon steel standards. Its wide application is in the automotive industry where it is used for bolts, studs, connecting rods, steering arms, etc. It is an ideal reference standard for the control of carbon, manganese, phosphorus, sulfur and silicon analyses. Data are presented in table 27.

Table 27. Certified carbon steel—SRM 20g.

Element	No. Det'ms	Method	Average %
Carbon	18	Thermal conductivity	0.457
Manganese	14	Persulfate-arsenite	.666
Phosphorus	8	Molybdenum-blue photometric	.011
Sulfur	18	Combustion-titration	.028
Silicon	8	Perchloric-acid dehydration	.308
Copper	4	Diethyldithiocarbamate photometric	.034
	12	Atomic absorption	.033
Nickel	4	Dimethylglyoxime photometric	.034
Molybdenum	8	Thiocyanate photometric	.007
Aluminum	12	Atomic absorption	.040

3. Stainless Steel-SRM 101f

This low carbon stainless steel contains 18 percent chromium and 10 percent nickel and was prepared by a pre-alloyed powder metallurgical process which included argon atomization and hydrogen annealing. This standard was prepared primarily for use in the steel industry for standardizing chemical methods of analysis particularly for carbon, sulfur, chromium and nickel. The data are presented in table 28.

4. Low Carbon Stainless Steel-SRM 166c

This special low-carbon stainless steel is provided as a control standard for very low levels of carbon. Newer instrumentation, such as thermal conductivity, now provides means for detecting very low levels of carbon in ferrous

Table 28. Certified stainless steel—SRM 101f.

Element	No. Det'ms	Method	Average %
Carbon	20	Thermal conductivity	0.014
Manganese	12	Activation analysis (Sec. 9)	.090
	8	Periodate photometric	.085
Phosphorus	8	Molybdenum-blue photometric	.007
Sulfur	12	Combustion-titration	.008
Silicon	8	Perchloric acid dehydration	.876
Copper	12	Activation analysis (Sec. 9)	.029
	4	Spark source isotope dilution (Sec. 6)	.030
Nickel	4	Dimethylglyoxime gravimetric	9.96
	8	Spark source isotope dilution (Sec. 6)	9.97
Chromium	4	Peroxydisulfate oxidation $FeSO_4$ titration	18.52
	8	Coulometric titration (Sec. 5)	18.47
Vanadium		HNO_3 oxidation-$FeSO_4$ titration	0.034
Molybdenum	12	Activation analysis (Sec. 9)	.007
Cobalt	11	Activation analysis (Sec. 9)	.087

alloys. This standard will serve as a bench-mark for standardizing and calibrating the many new types of instruments especially adapted for low carbon determinations. Using a thermal conductivity method, the carbon content based on 18 determinations was 0.0077 percent.

5. Differential Thermal Analysis Standards-SRM 755 and 756

These two compounds are the first materials that were made available for use as DTA standards. This work was undertaken with the cooperation of the Standards Committee of the International Conference of Thermal Analysis (ICTA). It is anticipated that additional materials will be investigated for use as DTA and DSC standards covering subambient to very high operating temperatures. Additional details were presented in a previous section.

a. Standard 755-SiO_2 (Quartz). The phase transition of this material is at approximately 575 °C and is the mean of the values obtained on several differential thermal analysis instruments. Generally, the differential temperature curve value may be somewhat higher than the adiabatic value and may vary for different equipment and heating rates. The standard is furnished in 2-gram units sized from 100 to 325 mesh.

b. Standard 756-KNO_3. The phase transition of this material is approximately 130 °C, and, like the quartz standard, is the mean of the values obtained on several differential thermal analysis instruments. The standard is furnished in 5-gram units as fine crystals.

6. Metallo-organic Standard-SRM 1061c Magnesium Cyclohexanebutyrate

This is one of a series of standards that were developed for use by the railroad and trucking industries as a basis for maintenance schedules and predictions of equipment failures. The amount of wear and the probability of failure of an internal combustion engine may be determined by analyzing its lubricating oil for metals. These analyses are made by optical emission spectroscopy. The certification of the magnesium was, however, determined by the gravimetric method as $Mg_2P_2O_7$. The value of magnesium based on 12 determinations was 6.45 percent.

7. Special Steels-SRM 361, 362 and 365

These 3 standards are part of a series of 5 graded steels that will eventually be certified for approximately 35 elements. However, the initial certification will be based on C, Mn, P, S, Si, Cu, Ni, Cr, V and Mo.

Standard 361 is a 0.4 carbon steel, grade AISI 4340. This material is being analyzed cooperatively by four industrial laboratories.

Standard 362 is a low alloy steel (0.15C), grade AISI 94B17, containing small amounts of tungsten, cobalt, niobium, tantalum and zirconium. This material is being analyzed cooperatively by 4 industrial laboratories.

Standard 365 is electrolytic iron containing very low levels of all other elements, except nickel, which is about 0.04 percent. Provisional certification includes nickel, in addition to 12 elements whose values are all <0.008. Later additional elements will be determined for inclusion on a final certificate.

R. K. Bell
J. R. Baldwin
E. R. Deardorff
T. C. Rains
T. A. Rush
J. T. Sterling
S. A. Wicks

8. PERSONNEL AND ACTIVITIES

A. Personnel Listing

Oscar Menis, Section Chief
James I. Shultz, Assistant Section Chief
Mary B. Pantazis, Section Secretary

J. R. Baldwin
R. K. Bell
D. S. Bright (part time)
R. W. Burke
E. R. Deardorff
R. Mavrodineanu
B. E. McClellan (summer)
T. C. Rains
T. A. Rush
J. T. Sterling
R. A. Velapoldi
S. A. Wicks

B. Publications

1. M. Tsutsui, R. A. Velapoldi, K. Suzuki, F. Vohwenkel, M. Ichikawa and T. Koyans, "Unusual Metalloporphyrins IV. Novel Methods for Metal Insertion into Porphyrins", J. Am. Chem. Soc 91, 6262-66 (1969).

2. W. Snelleman, T. C. Rains, K. W. Yee, H. D., Cook and O. Menis, "Flame Emission Spectrometry with Repetitive Optical Scanning in the Derivative Mode", Anal. Chem. 42, 394, 1970.

3. R. W. Burke and E. R. Deardorff, "Simultaneous Spectrophotometric Determination of Cobalt, Nickel and Copper with 2,3-Quinoxalinedithiol", Talanta 17, 255 (1970).

4. R. W. Burke, "Exchange Reactions of Ternary Ion Association Complexes Directly in the Organic Phase", Talanta 17, 240 (1970).

5. R. J. Kline and R. A. Velapoldi, "The Kinetics of the Acid Hydrolysis of trans Nitroaquobis(2,4-pentanedionato)cobalt(III), Inorg. Chem. 9, 1312-14 (1970).

6. O. Menis and B. E. McClellan, "Kinetics and Mechanism of Extraction of Iron(III) with β-Isopropyltropolone, submitted for publication to Analytical Chemistry.

7. O. Menis, B. E. McClellan and D. S. Bright, "Determination of the Formation Constants of Iron(III) and

Vanadium(V) with β-Isopropyltropolone Using the Extraction Method"; submitted for publication to Analytical Chemistry.

8. R. Mavrodineanu, "Sprayer and Burner Units for Flame Emission and Atomic Absorption Measurements", Develop.iin Applied Spectry 8, (1970); in print.

C. Talks

1. R. Mavrodineanu, "Sprayer and Burner Units for Flame Emission and Atomic Absorption Measurements", Mid-America Symposium on Spectroscopy, Chicago, Ill., May 1969.

2. T. C. Rains, "Atomic Absorption Spectroscopy", ACS Short Course on Atomic Absorption Spectroscopy, Dartmouth College, Hanover, New Hampshire, July 6-7, 1969.

3. R. Mavrodineanu (presented by T. C. Rains), "Sprayer and Burner Units for Flame Emission and Atomic Absorption Measurements", International Atomic Absorption Spectroscopy Conference, Sheffield, England, July 14-18, 1969.

4. T. C. Rains, "Applications of Atomic Absorption Spectrometry to the Determination of Major Constituents", Atomic Absorption Workshop, Center for Professional Advancement, Morristown, N. J., Aug 25-27, 1969.

5. T. C. Rains, "Atomic Absorption and Flame Emission Spectrometry—General Theory and Methodology", Southeastern Section of the Society for Applied Spectroscopy, Knoxville, Tennessee, Sept. 6, 1969.

6. T. C. Rains, "Future Trends in Atomic Absorption Spectrometry", Anachem Conference, Detroit, Michigan, Sept. 16-18, 1969.

7. T. C. Rains, "Atomic Absorption Spectroscopy", ACS Short Course on Atomic Absorption Spectroscopy, Washington, D. C., Oct. 25-26, 1969.

8. T. C. Rains and O. Menis, "AC Scanning in the Derivative Mode", Eastern Analytical Symposium, New York, N. Y., Nov. 19-21, 1969.

9. T. C. Rains, "Special Applications and Future Developments of Atomic Absorption Spectrometry", Baltimore-Washington Section of the Society of Applied Spectroscopy, W. R. Grace Research Center, Clarksville, Md., Dec. 16, 1969.

10. R. A. Velapoldi, "Induced Oxidation of Metalloporphyrins by Unsaturated Hydrocarbons", Oslo University Rikshospitalet, January, 1970.

11. O. Menis, "Determination of the Formation Constants of Iron(III) and Vanadium(V) with β-Isopropyltropolone Using Extraction Method", 159th American Chemical Society Meeting, Houston, Texas, Feb. 23, 1970.

12. O. Menis and B. E. McClellan, "Spectrophotometric Determination of Vanadium in Steels as the β-Isopropyltropolone Complex", Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1-6, 1970, Cleveland, Ohio.

13. T. C. Rains, T. A. Rush and O. Menis, "Selection of the Appropriate Absorbing and Non-Absorbing Line for Use in Atomic Absorption Spectrometry", Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1-6, 1970, Cleveland, Ohio.

14. R. W. Burke and E. R. Deardorff, "Simultaneous Spectrophotometric Determination of Cobalt, Nickel and Copper with 2,3-Quinoxalinedithiol, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1-6, 1970, Cleveland, Ohio.

15. Radu Mavrodineanu, "Burner and Supplying Systems for the Determination of Sodium and Potassium in Microliter Sample Volumes by Flame Spectrophotometry, Fifth Annual Meeting of the Association for the Advancement of Medical Instrumentation, Boston, Mass., March 23, 1970.

16. R. W. Burke, "The Use of Solutions for Checking the Absorbance Scales of Spectrophotometers", Fifth Annual

Meeting of the Association for the Advancement of Medical Instrumentation, Boston, Mass., March 23, 1970.

17. O. Menis, "Status of Thermal Analysis Standards", ASTM-NBS Symposium on Current Status on Thermal Analysis, NBS, April 20, 1970.

D. Committee Activities

Oscar Menis

Member ASTM, Committee E-3, Div. I., Thermal Analytical Test Methods Subcommittee on Standards.

Member ASTM, Committee E-10, Subcommittee I.

Editorial Board Member, Chemical Instrumentation, A Journal of Experimental Techniques in Chemistry and Biochemistry.

Institutional Representative, Standards Committee, International Confederation on Thermal Analysis.

Participant, National Committee Clinical Laboratory Standards (NCCLS) - Instrumentation.

Titular member of the International Union of Pure and Applied Chemistry Commission V3 on Analytical Nomenclature.

Referee, "Critical Review in Analytical Report".

James I. Shultz

Member ASTM, Advisory Board, Committee E-3.

Member ASTM, Vice Chairman, Div. F., Committee E-3.

Member ASTM, Secretary Editorial Subcommittee, Committee E-3.

Member ASTM, Committee E-3, Div. M.

Member ASTM, Committee E-3, Div. G.

Coeditor, ASTM Handbook of Methods of Chemical Analysis of Metals.

U. S. Delegate to ISO, Technical Committee 17, Subcommittee 1.

Chairman, Auditing Committee, Washington Professional Chapter, Alpha Chi Sigma.

T. C. Rains

American Chemical Society, Committee on Reagents Specifications, Subcommittee on Atomic Absorption.

Member ASTM, Committee E-2, Subcommittee X.

J. T. Sterling

Member ASTM, Chairman, Div. I., Committee E-3.

Member ASTM, Committee E-1, Thermoanalytical Test Methods.

R. K. Bell

Member ASTM, Committee E-3, Div. N.

Member Auditing Committee, Washington Professional Chapter, Alpha Chi Sigma.

E. R. Deardorff

Member ASTM, Task Force Chairman, Div. F., Committee E-3.

R. W. Burke

Member National Committee Clinical Laboratory Standards (NCCLS) - Instrumentation (UV-Vis Spectrophotometry).

Radu Mavrodineanu

Technical Coordinator of the Professional Development Program for Scientists Society for Applied Spectroscopy, Fall Session 1969, Baltimore-Washington.

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