NBS SPECIAL PUBLICATION 260-54

U.S. DEPARTMENT OF COMMERCE / National Bureau of Standards

Standard Reference Materials:

CERTIFICATION AND USE OF ACIDIC POTASSIUM DICHROMATE SOLUTIONS AS AN ULTRAVIOLET ABSORBANCE STANDARD—SRM 935

Standard Reference Materials:

Certification and Use of Acidic Potassium Dichromate Solutions as an Ultraviolet Absorbance Standard—SRM 935

R. W. Burke and R. Mavrodineanu

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

U.S. DEPARTMENT OF COMMERCE, Juanita M. Kreps, Secretary
Dr. Sidney Harman, Under Secretary
Jordan J. Baruch, Assistant Secretary for Science and Technology
NATIONAL BUREAU OF STANDARDS, Ernest Ambler, Acting Director

Issued August 1977

Library of Congress Catalog Card Number: 77-600027

National Bureau of Standards Special Publication 260-54

Nat. Bur. Stand. (U.S.), Spec. Publ. 260-54, 157 pages (Aug. 1977)

CODEN: XNBSAV

U.S. GOVERNMENT PRINTING OFFICE
WASHINGTON: 1977

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402
Price $3—Stock No. 003-003-01828-7

PREFACE

Standard Reference Materials (SRM's) as defined by the National Bureau of Standards are "well-characterized materials, produced in quantity, that calibrate a measurement system to assure compatibility of measurement in the nation." SRM's are widely used as primary standards in many diverse fields in science, industry, and technology, both within the United States and throughout the world. In many industries traceability of their quality control process to the national measurement system is carried out through the mechanism and use of SRM's. For many of the nation's scientists and technologists it is therefore of more than passing interest to know the details of the measurements made at NBS in arriving at the certified values of the SRM's produced. An NBS series of papers, of which this publication is a member, called the NBS Special Publication - 260 Series is reserved for this purpose.

This 260 Series is dedicated to the dissemination of information on all phases of the preparation, measurement, and certification of NBS-SRM's. In general, much more detail will be found in these papers than is generally allowed, or desirable, in scientific journal articles. This enables the user to assess the validity and accuracy of the measurement processes employed, to judge the statistical analysis, and to learn details of techniques and methods utilized for work entailing the greatest care and accuracy. It is also hoped that these papers will provide sufficient additional information not found on the certificate so that new applications in diverse fields not foreseen at the time the SRM was originally issued will be sought and found.

Inquiries concerning the technical content of this paper should be directed to the author(s). Other questions concerned with the availability, delivery, price, and so forth will receive prompt attention from:

Office of Standard Reference Materials
National Bureau of Standards
Washington, D.C. 20234

J. Paul Cali, Chief
Office of Standard Reference Materials

FOREWORD

Spectrophotometry is a technique widely used in analytical chemistry. Due to its inherent characteristics of high sensitivity and good specificity it has been applied to the establishment of fundamental parameters, as well as to the determination of the composition of inorganic or organic compounds in solids, liquids, or gases in science and industry. For example, in one field of application, clinical analytical chemistry, spectrophotometry is used in over 50 percent of the analytical methods commonly employed. The number of spectrophotometric analyses performed in an active hospital laboratory exceeds 1,000 measurements per day, with more than 1,000,000 measurements performed in the clinical laboratories in the U.S. daily. A conservative projection for the future indicates that a growth rate of 15 percent per year in spectrophotometric measurements in clinical analysis should be expected.

The widespread use of spectrophotometric techniques on samples vital to the health, safety, and industry of the nation makes it imperative that suitable means be available to establis the accuracy of these analyses. Because of the importance of these measurements, the Analytical Chemistry Division of the Institute for Materials Research is conducting an extensive program to study and develop means which can be used by the analytical chemist to verify the proper functioning of spectrophotometers, and to insure that the data produced are reliable.

A principal ingredient in the Analytical Chemistry Division program has been the design and construction of a high-accuracy spectrophotometer which represents the primary optical transmittance standard. This instrument was used to produce a number of transfer Standard Reference Materials in the form of neutral glass filters and liquid filters certified for transmittance at various wavelengths and transmittance levels. Quartz cuvettes with certified paralellism and pathlength were also produced at NBS.

This Special Publication describes another transmittance/absorbance transfer standard which has been developed within the program. This material, crystalline potassium dichromate, allows certification to be extended into the ultraviolet spectral region and, additionally, provides a convenient means to verify the linearity of the absorbance scale of conventional spectrophotometers. This material is now available as Standard Reference Material 935. The details of the selection, preparation, certification, and use of this SRM are described in detail in this publication.

Improved methods and materials which can be used to verify the proper functioning of conventional spectrophotometers are now being actively investigated. Examples are evaporated metal-on-fused silica filters, which should extend the transmittance/absorbance calibration range from 200 nm to 3000 nm, and liquid materials, such as holmium perchlorate solutions, which can be used for verifying the accuracy of the wavelength scale of spectrometers in transmittance and luminescence measurements.

The availability of these Standard Reference Materials, and their use by the analyst, should contribute significantly to accurate measurements in spectrophotometry and should provide a solid base for future progress in this field.

P. D. LaFleur, Chief
Analytical Chemistry Division

TABLE OF CONTENTS

LIST OF TABLES

TABLE NO. PAGE

LIST OF FIGURES

CERTIFICATION AND USE OF ACIDIC POTASSIUM DICHROMATE SOLUTIONS AS AN ULTRAVIOLET ABSORBANCE STANDARD

R. W. Burke and R. Mavrodineanu

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

The apparent specific absorbances of 0.001N perchloric acid solutions of a high purity potassium dichromate salt, Standard Reference Material (SRM) 935, have been certified at four wavelengths in the ultraviolet—235, 257, 313 and 350 nm. This publication describes in detail the measurements leading to the certification, and discusses the use of this SRM as a transfer standard for verifying the accuracy of the absorbance scale of narrow bandpass absorption spectrometers in this important wavelength region. Apparent specific absorbance data are also reported near two predicted isosbestic wavelengths in the acidic $HCrO_4^-/Cr_2O_7^=$ system. The apparent specific absorbances at 345 nm are sufficiently independent of concentration that absorbance measurements at this wavelength can be used for verifying absorbance linearity to about one part in a thousand.

Key Words: Absorbance linearity; accuracy of transmittance or absorbance scale; acidic potassium dichromate solutions; calibration of ultraviolet spectrometers; certification of apparent specific absorbances; isosbestic wavelengths; liquid filters; Standard Reference Material; transfer standards.

1. GENERAL

The objective of this work was to provide the analyst with a Standard Reference Material (SRM) which can be used in laboratory practice to verify the accuracy of the transmittance or absorbance scale of conventional spectrometers in the ultraviolet region of the spectrum. The material selected for this purpose was a potassium dichromate salt from which the user must prepare adequate solutions in 0.001N perchloric acid. The values given in this work, and in the subsequent certificate of calibration, are derived from absorbance measurements which were not corrected for multiple internal reflections within the cuvette and for buoyancy effects. The option was believed justifiable in the use of this SRM as a transfer standard since, in practice, internal reflections are common to all measurements in spectrometers, and the variation of buoyancy from one location to another is negligible. Hence, the specific absorbances[1] certified do not define an absolute physical parameter of this material. These values are therefore termed apparent specific absorbances, ε_a, and are used throughout this work and in the certificate to signify that they have not been corrected for these two effects. These values, however, allow the use of this SRM as a transfer standard and do not require additional data manipulation by the user. This variance from the strict definition of specific absorbance, ε, was made for convenience but still permits the transfer to the user the accuracy and traceability derived from direct measurements on the National Bureau of Standards high accuracy transmission spectrometer. The reader interested in the specific absorbances of this material should consult reference 9 in which the corrected

---

[1] The spectrometry nomenclature used in this publication is defined in a recent article by K. D. Mielenz. A copy of this paper is enclosed in the Appendix.

values are given. The difference between the corrected and uncorrected values does not exceed 0.2 percent.

2. INTRODUCTION

During the past forty years molecular absorption spectrometry has been a much used analytical technique in all areas of science and technology. Despite this widespread use there has been, until recently, no suitable Standard Reference Material available for verifying the accuracy of the transmittance or absorbance scales of the spectrometers on which the measurements were made. Yet, as early as the late 1940's, the question of photometric accuracy was of general concern. Soon thereafter the results of various collaborative testings of absorption spectrometers began appearing in the literature. Among the most notable of these were the tests conducted by the British Photoelectric Spectrometry Group [1,2], by a similar Dutch Group [3] and by Vandenbelt [4,5] in the United States. All of these tests showed significant differences between measurements made on different instruments. However, since no certified transmittance or absorbance standards were available to assess accuracy, the only other alternative was to use agreement with consensus results in order to intercompare instruments.

This situation remained essentially unchanged until 1969 when, largely from requests by the American Association of Clinical Chemists and the College of American Pathologists, a program was initiated at the National Bureau of Standards (NBS) in the Analytical Chemistry Division of the Institute for Materials Research. The express purpose of this program was to develop and make available to the public Standard Reference Materials which could be used to establish that an absorption spectrometer was functioning properly and was producing meaningful and reliable data. With partial funding from the National Institute of General Medical Sciences, this program led, in 1970, to the design and construction of a high-accuracy transmission spectrometer [6,7]. This instrument, in turn, was used to develop and certify two Standard Reference Materials (SRM's) — SRM 930, Glass Filters for Spectrophotometry, and SRM 931, Liquid Filters for Spectrophotometry. These SRM's were issued in 1971. A third Standard Reference Material, SRM 932, Quartz Cuvettes for Spectrophotometry, was added in 1973 and was produced in association with the Optical Physics Division of the Institute for Basic Standards at NBS. SRM 932 was intended to be used in association with SRM 931, and in general, in all measurements requiring accuracy in determining the transmittance or absorbance of liquids.

Since the issuance of these Standard Reference Materials, research interest has focused on developing new SRM's which can be used in the ultraviolet. The need for such standards has been voiced again most strongly by the clinical chemists and pathologists. They emphasize the importance of these SRM's by pointing out, for example, that progress in all areas of enzyme research and analysis is directly related to the accuracy of absorbance measurements made in the wavelength range from 260 to 350 nm. Neither of the two current SRM's meet this need. The glass filters do not transmit below about 350 nm and the liquid filters are certified at only one wavelength in the ultraviolet, 302 nm.

Progress in this research effort was described in two papers presented at the Workshop Seminar on Standardization in Spectrophotometry and Luminescence Measurements held in the NBS facilities at Gaithersburg, Maryland, on November 19-20, 1975. These papers have since been published in the NBS Journal of Research [8,9]. The first paper described an extensive evaluation of the solid metal-on-quartz type filters while the second presented data which showed that acidic potassium dichromate solutions were the most promising liquid standards that we had studied thus far for use in the ultraviolet.

Following that meeting, the decision was made to certify and issue a high purity potassium dichromate salt in solid form (SRM 935), to be used as an SRM for verifying the accuracy of the absorbance scale of narrow bandpass spectrometers over the range 235-350 nm. In as much as this SRM is being issued as a solid, it is being certified for its apparent specific absorbances which have been determined under a set of selected and well-defined conditions. In practice, the user will have to prepare solutions containing known concentrations of the SRM and, using the Beer-Lambert equation, convert the certified apparent specific absorbance data into accurate absorbance or transmittance values.

This publication describes in detail the operations involved in the certification of SRM 935 and demonstrates, with examples, its function as a transfer standard for verifying the accuracy of the transmittance or absorbance scales of narrow bandpass absorption spectrometers. Reprints of papers pertinent to the subject matter discussed in this report have been reproduced and included in the Appendix Section for the convenience of the reader.

3. SELECTION OF POTASSIUM DICHROMATE AS AN ULTRAVIOLET ABSORBANCE STANDARD

The decision to certify and recommend acidic potassium dichromate solutions as transfer standards for verifying the accuracy of the transmittance or absorbance scale of narrow bandpass absorption spectrometers in the ultraviolet was made after extensive studies had shown this system to be the best of those liquid filters considered for this purpose. Other systems considered potentially useful included potassium nitrate, potassium acid phthalate, benzoic acid, p-nitrophenol, alkaline potassium chromate, composite solutions of metal ions, and various water-soluble organic dyes. These studies have been described previously [10]. Since both the acidic and alkaline chromium systems were clearly superior to the others in terms of the spectral range covered, the final choice was reduced to choosing between these two. Subsequently, the acidic system was selected because of the following considerations: (a) more useful distribution and relation of absorbance maxima and minima, (b) more favorable weighing factor, (c) a smaller temperature dependence, (d) less reaction with glassware, and (e) greater ease of transferring and rinsing. These advantages were considered to more than offset the one disadvantage that can be cited for the acid potasssium dichromate system, namely that the absorbance, as a function of concentration, exhibits a stronger deviation from linearity than most systems. As will be shown in subsequent sections of this report, this deviation detracts little, if any, from the practical use of this material as a transfer standard.

4. IONIC COMPOSITION AND SPECTRAL PROPERTIES OF POTASSIUM DICHROMATE SOLUTIONS

The ionic composition of aqueous chromium (VI) solutions is dependent on their acidity or alkalinity. Variation of composition with pH was discussed in detail in a previous paper [10]. A copy of this publication is included in the Appendix. In alkaline solution chromium (VI) exists only in the form of $CrO_4^=$ ions. In weakly acidic media, the predominant chromium (VI) ion is $HCrO_4^-$. In addition, and depending on the total concentration of chromium present, there exist varying amounts of a second ion, $Cr_2O_7^=$. This ion is formed by the dimerization of $HCrO_4^-$. The equilibrium expression for this dimerization may be written as follows:

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

where K_D, the concentration dimerization constant, is given by

$$K_D = \frac{[Cr_2O_7^=]}{[HCrO_4^-]^2} \qquad (2)$$

Using the value of K_D determined previously [10], it can be shown that, for the range of potassium dichromate solutions used in this study, the percentage of the total chromium (VI) present as the $Cr_2O_7^=$ dimer varies from about one to four percent.

The variation in the ionic composition with concentration is the principal cause of the observed deviations of the acid system from Beer's law. These deviations are easily measured at those wavelengths where the specific molar absorbances of the $Cr_2O_7^=$ and $HCrO_4^-$ ions are different. As shown in figure 1, at most wavelengths in the ultraviolet the $Cr_2O_7^=$ ion is more strongly absorbing than the $HCrO_4^-$ form. Consequently, at these wavelengths, solutions would be expected to exhibit increasingly positive deviations from Beer's law with increasing chromium concentration. The reverse behavior, but to a lesser degree, would be expected for those wavelengths in the range of approximately 320-345 nm since, in this region, the $Cr_2O_7^=$ ion is the less strongly absorbing form. At the two isosbestic points both ions have the same specific molar absorbances and no deviations from changes in ionic composition would be expected. The data presented in the following section will demonstrate better these various effects.

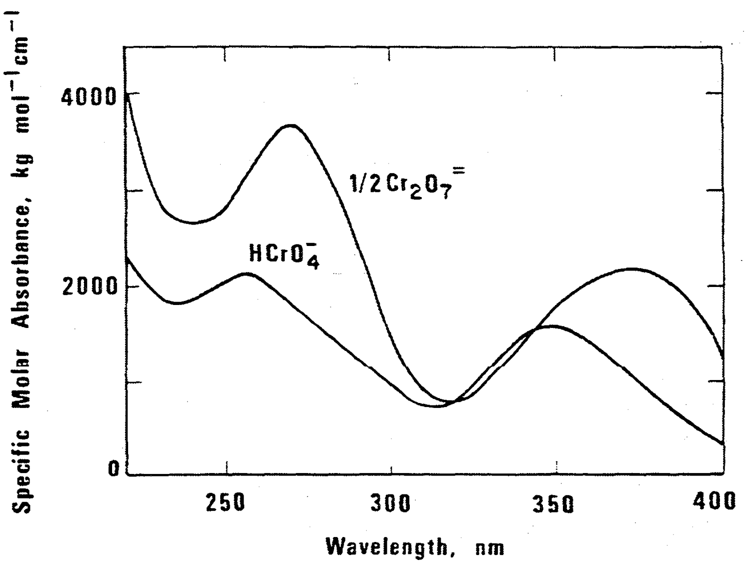


Figure 1. Absorbance spectra of the $HCrO_4^-$ ion and its dimerization product, $Cr_2O_7^=$.

5. MEASUREMENT OF THE APPARENT SPECIFIC ABSORBANCES OF SRM 935

5.1 Source and Purity of the Potassium Dichromate SRM

The potassium dichromate ($K_2Cr_2O_7$) salt used for this SRM was an analytical reagent grade material purchased as a single lot from the J. T. Baker Chemical Company,[2] Phillipsburg, New Jersey 08865. Drying studies performed at 105 °C showed that the surface moisture of this material was less than 0.01 percent. No attempts were made to determine possible occluded water. However, a recent paper [11] by

---

[2] In order to describe materials and experimental procedures adequately, it is occasionally necessary to identify the sources of commercial products by the manufacturer's name. In no instance does such identificaton imply endorsement by the National Bureau of Standards, nor does it imply that the particular product is necessarily the best available for that purpose.

Yoshimori and Sakaguchi has shown that $K_2Cr_2O_7$ typically contains 0.01 to 0.02 percent moisture which can be removed only by crushing and drying at temperatures in excess of 350 °C. SRM 935 is intended to be used without further drying. When not in use, it is recommended that it be capped and stored in a dessicator over magnesium perchlorate to avoid undue exposure to humidity.

A coulometric assay of the purity of the undried material was performed in the Analytical Chemistry Division by G. Marinenko. The purity of SRM 935, expressed as an oxidant, was found to be 99.972 ± 0.005 percent where the uncertainty figure represents the 95 percent confidence interval for the mean based on 11 degrees of freedom. In addition, this material was examined for trace elemental impurities by J. A. Norris, also from the Analytical Chemistry Division, using emission spectrometry. The only significant impurities detected were sodium and rubidium. Their concentrations were estimated to be in the range 0.02 and 0.03 percent, respectively.

5.2 Preparation of Solutions

All concentrations of potassium dichromate solutions used in this study were prepared, and are expressed, on a weight basis. Each solution was prepared individually by weighing milligram amounts of the $K_2Cr_2O_7$ SRM to the nearest microgram on a sensitive microbalance. The weighed samples were transferred quantitatively to 1-liter volumetric flasks which had been pre-weighed to the nearest 0.01 g on a single-pan, top-loading balance. The samples were dissolved in 400-500 ml of distilled water shown to be free of reducing impurities (see Section 6.7 for recommended test) and 1 ml of 1N perchloric acid, prepared from reagent grade material, was added to each to adjust acidity. After diluting approximately to volume with distilled water, the flasks were sealed with tightly-fitting, double-ribbed Teflon stoppers to prevent evaporation and the solutions were mixed well. These stoppers are available from Kontes Glass Company, Vineland, New Jersey 08360. The flasks and contents were weighed again on the top-loading balance to the nearest 0.01 g and the net weights of the solutions determined by difference. The concentrations of each solution were then calculated and expressed as mg $K_2Cr_2O_7$/kg of solution. A solvent blank was prepared by diluting 1 ml of 1N perchloric acid to 1 liter with distilled water.

Prior to preparing the $K_2Cr_2O_7$ solutions, the accuracy of the two balances was checked with weights calibrated in the Mass Section of the NBS Institute for Basic Standards. Table 1 shows the corrections that were applied to all weighings made on the microbalance. No correction was found necessary for weighings made on the top-loading balance.

Table 1. Calibration of the microbalance.

Nominal Wt. mg	True Wt. mg	Measured Wt. mg	Correction mg
20	19.9993	20.002	-0.003
40	39.9932	39.995	-0.002
60	59.9941	60.001	-0.007
80	79.9798	79.976	+0.004
100	99.9924	99.992	0.000

5.3 Instrumentation

The Institute for Materials Research high accuracy transmission spectrometer was used for performing all transmittance measurements which, in turn, were converted to absorbance by an appropriate computer program. The design and construction of this instrument have been described in detail by one of us (RM) in reference 7 and will not be repeated here. Similarly, the quartz cuvettes used are also of NBS design and construction and have been described previously [12]. These certified cuvettes are identified as SRM 932 and are available through the NBS Office of Standard Reference Materials. A copy of the paper describing the construction and calibration of these cuvettes is included in the Appendix.

5.4 Selection of Wavelengths

Four wavelengths — 235, 257, 313 and 350 nm — were chosen for the certification of the apparent specific absorbances of acidic potassium dichromate. These wavelengths are indicated in the absorbance spectrum shown in figure 2. They were selected because they are least subject to small inaccuracies that may be present in the wavelength scales of the instruments for which this SRM is intended. Although the spectral bandpass effect has been increased at these wavelengths in comparison to measurements made along the slopes of the spectrum, the restrictions are not severe. The bandwidths of the 257 and 350 nm peaks are approximately 40 and 60 nm respectively and therefore an instrumental spectral bandwidth of 2 nm or less is sufficent for obtaining at least 0.999 of the maximum peak intensities (see also pp. 30 to 32).

In addition to the four above-mentioned wavelengths chosen for certification of apparent specific absorbances, four additional wavelengths were selected in each of the regions of the two isosbestic points shown in figure 1. This approach was necessary because of the uncertainty in the $Cr_2O_7^=$ spectrum which is a calculated spectrum. The details of its derivation have been described in reference [10]. The four wavelengths chosen as the best estimates of these isosbestic points were 322, 323, 345 and 346 nm.

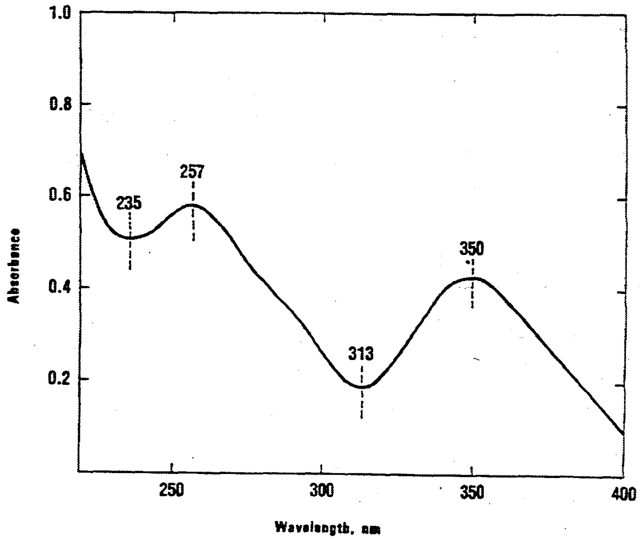


Figure 2. Absorbance spectrum of a 40 mg·kg^{-1} solution of $K_2Cr_2O_7$ in 0.001N perchloric acid.

5.5 Details of the Transmittance/Absorbance Measurements

All of the transmittance/absorbance measurements were made relative to air in a temperature-controlled room at 23.5 ± 0.2 °C. Two sets of measurements were necessary therefore in order to determine the absorbance required for the calculation of an apparent specific absorbance. These were: A measurement of the transmittance density, $D = -\log_{10}T$, of the solvent blank (D_B) and a similar measurement of the sample solution (D_S). The apparent absorbance (A_a) due to the chromium (VI) ions was then computed from the relation $A_a = D_S - D_B$.

The first transmittance/absorbance measurements performed on the high-accuracy transmission spectrometer entailed making a series of runs on a solvent blank. Five of the certified cuvettes described in reference 12 (reproduced in the Appendix) were selected for this experiment. This number was chosen because, in subsequent experiments, five different concentrations of potassium dichromate were to be measured.

The cuvettes were cleaned by immersion in concentrated (36N) sulfuric acid for several hours. They were then transferred consecutively to 24, 12 and 6N solutions of sulfuric acid in order to eliminate excessive generation of heat upon rinsing directly with distilled water and thereby avoid the possibility of deforming them. They were then allowed to air-dry under an inverted Petri dish. Finally, the windows of the cuvettes were wiped with a lint-free lens tissue immediately before placing in the spectrometer. Once placed into their respective holders, they were not removed again until all the measurements necessary for the certification of SRM 935 had been completed.

Transfer of solvent and sample solutions to and from the cuvettes were made by means of 23-cm, borosilicate, Pasteur-type, disposable pipettes fitted with 2 ml rubber bulbs. These pipettes and bulbs are readily available from all major chemical supply houses. Similar pipettes made from soft glass are also widely available. The soft glass types are not recommended because, in general, their surfaces are contaminated with ultraviolet absorbing contaminants and they also have an alkaline surface which could be an additional source of error [13]. As a precautionary measure, the borosilicate pipettes were always rinsed thoroughly with distilled water and dried in an oven at 105 °C before using.

The pattern of results obtained for the five cuvettes when they were filled with blank solvent and their apparent absorbances measured relative to air is shown in figure 3. These initial measurements were made at 235 nm because it was the shortest wavelength of interest and any problems associated with cuvette cleanliness or transfer of solution were expected to be maximal at this wavelength. All of the cuvettes exhibit the same qualitative absorbance behavior upon sucessive fillings with solvent. The term "rinse" as used in figure 3 and throughout the remainder of this report refers to the exchange of one cuvette volume for a second

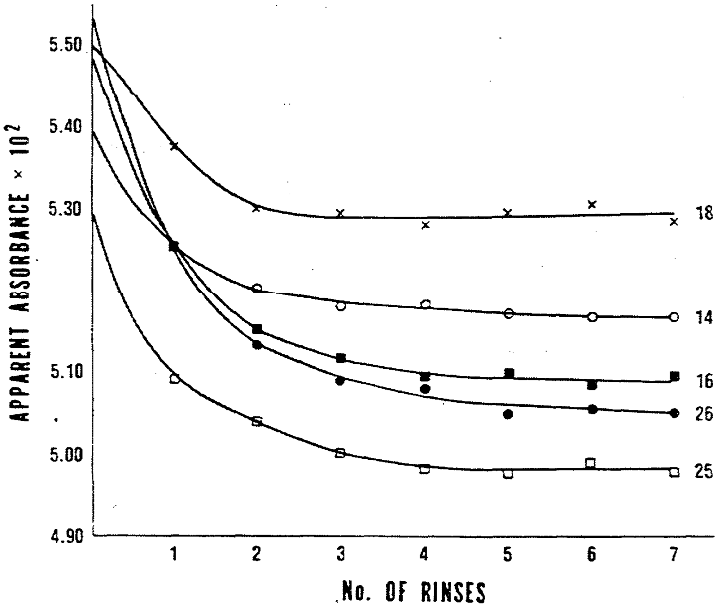


Figure 3. Apparent absorbances, A_a, of the blank solvent measured against air in the five cuvettes used—Nos. 14, 16, 18, 25, and 26; ref. [6].

volume. A minimum and constant absorbance reading was obtained in each case after 5-6 rinses. Consequently six rinses were used in the transfer of all blank and sample solutions. Following these measurements the first set of acidic potassium dichromate solutions were prepared. Comprising this set were six 0.001N perchloric solutions having nominal concentrations of 0, 20 40, 60, 80 and 100 mg $K_2Cr_2O_7$/kg. The $K_2Cr_2O_7$ solutions were prepared accurately by weight as described previously and immediately placed in the controlled-temperature room housing the high-accuracy transmission spectrometer and allowed to equilibrate. Generally, for convenience, the solutions were prepared one afternoon and measurements were made the following day. This series of measurements were begun by repeating the absorbance measurements on the solvent blank at 235 nm.

Since these values were in agreement with those obtained previously, similar blank measurements were then made at the other seven wavelengths of interest. Thereupon, the solvent was removed from the five cuvettes by means of the Pasteur-type, disposable pipettes and immediately replaced with the acidic potassium dichromate solutions. This operation was performed in a sequential manner and, as a matter of convention, the lowest $K_2Cr_2O_7$ concentration was always placed in the first cuvette and the highest concentration in the last. Six rinses of each cuvette were made with the appropriate sample solution in order to assure that the concentrations of the solutions measured were identical to those sampled.

Two different pipettes were used alternately for the filling and emptying operation associated with the transfer of each solution. After the third rinse, the pipette used originally for filling was made the emptying pipette and a new pipette was taken for the filling step. Such an approach appeared to be a good technique for avoiding concentration errors and thereby having a truly representative sample in the cuvette before the measurements were made. After the sixth rinse the last transfer was made as quickly as possible (approximately 10 s) and the cuvette was sealed with a Teflon stopper. This sequence of operations was repeated for each solution and cuvette combination until all five $K_2Cr_2O_7$ solutions were loaded into the high-accuracy spectrometer.

Three cycles of transmittance/absorbance measurements were then made on each of the solutions at the eight wavelengths selected. Each cycle was separated in time by one revolution of the rotating sample turn-table [7]. All results were presented on a teletype printout in the form illustrated in table 2. The measurements shown were made at 235 nm. A cuvette containing blank solvent was in position 2. The cuvettes containing the five acidic potassium dichromate solutions and having nominal concentrations of 20, 40, 60, 80 and 100 mg $K_2Cr_2O_7$/kg were in positions 4, 6, 8, 10 .

Table 2. Teletype printout of transmittance/absorbance data for a set of acidic $K_2Cr_2O_7$ solutions; λ = 235 nm.

```
 1  .200112E 01   .4423E-03
 2  .177946E 01   .2932E-03
 3  .200142E 01   .3373E-03   88.916   88.943    .0509
 4  .100215E 01   .7656E-03
 5  .200173E 01   .5061E-03   50.067   50.063    .3005
 6  .567963E 00   .6426E-03
 7  .200142E 01   .4768E-03   28.375   28.370    .5471
 8  .317861E 00   .1170E-02
 9  .200153E 01   .4663E-03   15.381   15.379    .7992
10  .173780E 00   .1354E-02
11  .200145E 01   .4398E-03    8.932    8.931   1.0491
12  .992660E-01   .2317E-02
13  .200247E 01   .5060E-03    4.958    4.958   1.3047
14  .552583E 00   .1093E-02
15  .200136E 01   .3479E-03   27.599   27.594    .5592
 1  .200142E 01   .4303E-03
 2  .177936E 01   .4063E-03
 3  .200119E 01   .4957E-03   88.910   88.936    .0509
 4  .100168E 01   .6841E-03
 5  .200126E 01   .5108E-03   50.053   50.048    .3006
 6  .563272E 00   .5973E-03
 7  .200176E 01   .4917E-03   28.392   28.386    .5469
 8  .317599E 00   .1235E-02
 9  .200116E 01   .3510E-03   15.368   15.366    .7995
10  .173859E 00   .1102E-02
11  .200073E 01   .3285E-03    8.939    8.937   1.0488
12  .992528E-01   .1390E-02
13  .200143E 01   .3783E-03    4.960    4.959   1.3046
14  .552138E 00   .6704E-03
15  .200071E 01   .3046E-03   27.592   27.586    .5593
 1  .200059E 01   .3715E-03
 2  .177765E 01   .4363E-03
 3  .199997E 01   .6911E-03   88.370   88.897    .0511
 4  .100156E 01   .7149E-03
 5  .200041E 01   .4241E-03   50.073   50.068    .3004
 6  .567657E 00   .5464E-03
 7  .200050E 01   .4131E-03   28.376   28.371    .5471
 8  .317523E 00   .8951E-03
 9  .199993E 01   .3614E-03   15.374   15.372    .7994
10  .178623E 00   .1333E-02
11  .200049E 01   .4893E-03    8.930    8.929   1.0492
12  .991913E-01   .1741E-02
13  .200025E 01   .5100E-03    4.959    4.958   1.3047
14  .552294E 00   .1126E-02
15  .200115E 01   .4190E-03   27.605   27.599    .5591

RUN NO. 170 NO. CYCLES  3 START LOC.  1 END LOC. 15 NO. PTS. 50
POS   AVERAGE CORR. T     AVERAGE ABSBNC

  2       88.925             .05097

  4       50.060             .30051

  6       28.375             .54705

  8       15.372             .79936

 10        8.932            1.04903

 12        4.958            1.30463

 14       27.593             .55919
```

NOTE: The printout contains the data from three sets of transmittance measurements made on seven samples (I) measured against air (I^o). The data from the first column identify the positions at which the measurements are performed. The odd numbers represent the eight I^o measurements which bracket the seven I measurements (even numbers). The second column gives the average of the 50 individual measurements of the photocurrent made in 2.5 s, and corresponding to I^o and I. Column 3 contains the standard deviation of the 50 individual measurements averaged in column 2. The non-corrected transmittance is given in column 4, which is deduced from:

$$\frac{I_2}{\frac{I_1^o + I_3^o}{2}} = T$$

The corrected transmittance is given in column 5 and the corresponding calculated transmittance density ($-\log_{10}T$) is given in column 6. The corresponding averages from the triplicate measurements are given at the bottom of the printout. For further details consult reference 7.

and 12, respectively. A filter consisting of inconel-on-quartz [8] was placed in position 14 for use as a reference.

In table 2, column 5 is the transmittance (T) relative to air after correction for detector non-linearity; column 6 contains the corresponding corrected transmittance density (D) calculated from the relation $D = -\log_{10}T$. At the bottom of table 2, the average values for corrected T and D are tabulated for the three cycles of measurements. From the data the apparent absorbance (A_a) was calculated using the relation $A_a = D_{sample} - D_{blank}$. Immediately upon completion of the first series of measurements for all eight wavelengths, the cuvettes containing the five sample solutions were rinsed twice with fresh solution, refilled and measured again. Invariably, since no systematic differences were observed between the sets of duplicate runs, the values were simply averaged and used for the subsequent calculation of apparent specific absorbances.

For the certification of SRM 935, six samples of each of the five concentrations of acidic potassium dichromate solutions were prepared and measured according to the procedure just described. All transmittance/absorbance measurements were made on a given set of solutions on the same day. Approximately three weeks were required to complete the measurements on the six sets.

5.6 Calculation of the Apparent Specific Absorbances of SRM 935 Potassium Dichromate in 0.001N Perchloric Acid

Specific absorbances were calculated at the eight wavelengths of interest for each of the 30 potassium dichromate solutions measured using the relation

$$\varepsilon_a = \frac{D_S - D_B}{b \cdot c} = \frac{A_a}{b \cdot c} \tag{3}$$

where:

ε_a = apparent specific absorbance
D_S = transmittance density of sample solution
D_B = transmittance density of the solvent blank
b = internal light path of the cuvette in centimeters
c = weight concentration of potassium dichromate solution in g kg^{-1}
A_a = apparent absorbance.

Before calculation of ε_a, the apparent absorbances, A_a, ($A_a = D_s - D_B$) were corrected for a small systematic error arising from the fact that the light beam in the IMR high-accuracy spectrometer is not collimated but instead is slightly convergent at the sample position. This correction has been mentioned by Mielenz and Mavrodineanu [14]. The error in absorbance, $\Delta A/A$, for normal incidence at the cuvette for this instrument was shown to be 6.3×10^{-4}. All values of A_a were subsequently corrected by multiplying by the factor 0.99937.

The exact values used for the internal light paths of the five sample cuvettes were the averages of the values given for positions seven through ten in table 2 of reference 12, a copy of which is included in the Appendix. These averages were the values used for b in Eq. (3).

As discussed previously, no corrections were made to the measured transmittances or absorbances for internal multiple reflections since this effect is present to the same extent, within the uncertainty of the measurements, in both the certification and the intended use of this SRM. Internal multiple reflections are not instrument dependent and therefore corrections for their effect are not necessary in order to certify and use this SRM as a transfer standard. On the other hand, if this material were being certified for specific molar absorbance, appropriate corrections for internal multiple reflections should be applied. Burnett [15] has discussed recently the magnitude of the internal multiple reflection correction as a function of wavelength and absorbance level. The reader should refer to this paper before attempting to make and report specific molar absorbance data. Potential corrections for reflections from other surfaces such as lenses and slit jaws [14] were disregarded also because they were believed adequately eliminated by use of the solvent blank.

Values of the apparent specific absorbances of SRM 935 potassium dichromate were calculated using Eq. (3) for the eight wavelengths chosen and for the 30 $K_2Cr_2O_7$ solutions measured. The only correction applied to the experimental data was the one for convergent beam geometry. The apparent specific absorbances calculated are summarized in Tables 3 and 4.

Table 3. Apparent specific absorbances, ε_a, calculated from transmittance/absorbance measurements on five concentration levels of SRM 935 potassium dichromate in 0.001N perchloric acid at wavelengths corresponding to absorbance maxima or minima; T = 23.5 °C.

		ε_a, kg·g^{-1}·cm^{-1}			
	$K_2Cr_2O_7$ Conc., mg kg^{-1}	235(1.2)^{a}nm	257(0.8)nm	313(0.8)nm	350(0.8)nm
		Min.	Max.	Min.	Max.
	20.346	12.254	14.249	4.801	10.668
	20.112	12.244	14.252	4.796	10.667
	20.269	12.264	14.268	4.802	10.676
	20.112	12.279	14.278	4.811	10.681
	20.136	12.264	14.264	4.806	10.674
	20.107	12.273	14.267	4.805	10.676
Av.	20.180	12.263	14.263	4.803	10.674
S.D.^{b}		0.013	0.011	0.005	0.005
	40.612	12.295	14.314	4.807	10.674
	40.292	12.302	14.320	4.816	10.685
	40.300	12.303	14.323	4.815	10.684
	40.333	12.301	14.311	4.815	10.682
	40.288	12.304	14.320	4.815	10.685
	40.354	12.307	14.315	4.814	10.677
Av.	40.363	12.302	14.317	4.814	10.681
S.D.		0.004	0.005	0.003	0.005
	60.561	12.343	14.368	4.812	10.685
	60.458	12.342	14.370	4.816	10.690
	60.426	12.348	14.379	4.817	10.690
	60.334	12.345	14.372	4.816	10.688
	60.338	12.349	14.375	4.815	10.690
	60.385	12.351	14.368	4.816	10.687
Av.	60.417	12.346	14.371	4.815	10.688
S.D.		0.004	0.004	0.002	0.002

Table 3 continued.

		ε_a, $kg \cdot g^{-1} \cdot cm^{-1}$			
	$K_2Cr_2O_7$ Conc., mg kg^{-1}	322(1.2)[a]nm	257(0.8)nm	313(0.8)nm	350(0.8)nm
		Min.	Max.	Min.	Max.
	80.534	12.390	14.439	4.820	10.702
	81.119	12.392	14.450	4.824	10.706
	80.528	12.397	14.450	4.823	10.705
	80.999	12.396	14.459	4.824	10.708
	80.442	12.392	14.448	4.821	10.704
	80.383	12.394	14.442	4.821	10.701
Av.	80.668	12.394	14.448	4.822	10.704
S.D.		0.003	0.007	0.002	0.003
	100.722	12.430	14.482	4.827	10.714
	100.845	12.434	14.488	4.825	10.714
	100.772	12.438	14.491	4.828	10.711
	101.081	12.434	14.481	4.826	10.710
	100.438	12.437	14.486	4.827	10.711
	100.420	12.438	14.477	4.822	10.710
Av.	100.713	12.435	14.484	4.826	10.712
S.D.		0.003	0.005	0.002	0.002

---

[a] Spectral bandpass.

[b] Standard deviation of a single determination.

Table 4. Apparent specific absorbances, ε_a, calculated from transmittance/absorbance measurements on five concentration levels of SRM 935 potassium dichromate in 0.001N perchloric acid at wavelengths near the two isosbestic points; T = 23.5 °C.

	$K_2Cr_2O_7$ Conc., mg kg^{-1}	ε_a, $kg \cdot g^{-1} \cdot cm^{-1}$ 322(0.8)[a]nm	323(0.8)nm	345(0.8)nm	346(0.8)nm
	20.346	5.855	6.070	10.606	10.621
	20.112	5.849	6.068	10.598	10.623
	20.269	5.860	6.078	10.607	10.623
	20.112	5.849	6.070	10.611	10.638
	20.136	5.843	6.068	10.609	10.630
	20.107	5.868	6.091	10.616	10.629
Av.	20.180	5.854	6.074	10.608	10.627
S.D.[b]		0.009	0.009	0.006	0.006
	40.612	5.845	6.071	10.597	10.621
	40.292	5.858	6.074	10.602	10.631
	40.300	5.859	6.071	10.601	10.629
	40.333	5.843	6.061	10.599	10.629
	40.288	5.841	6.064	10.596	10.624
	40.354	5.864	6.081	10.604	10.627
Av.	40.363	5.852	6.070	10.600	10.627
S.D.		0.010	0.007	0.003	0.004
	60.561	5.844	6.067	10.597	10.628
	60.458	5.846	6.064	10.597	10.630
	60.426	5.851	6.063	10.597	10.631
	60.334	5.836	6.057	10.593	10.628
	60.338	5.828	6.053	10.594	10.630
	60.385	5.854	6.074	10.597	10.628
Av.	60.417	5.843	6.063	10.596	10.629
S.D.		0.010	0.007	0.002	0.001

Table 4 continued.

		- - - - - - - - ε_a, kg·g^{-1}·cm^{-1} - - - - - - - -			
	$K_2Cr_2O_7$ Conc., mg kg^{-1}	322(0.8)^{a}nm	323(0.8)nm	345(0.8)nm	346(0.8)nm
	80.534	5.839	6.062	10.600	10.630
	81.119	5.843	6.062	10.602	10.637
	80.528	5.845	6.062	10.602	10.635
	80.999	5.833	6.055	10.602	10.636
	80.442	5.824	6.050	10.601	10.631
	80.383	5.850	6.071	10.606	10.634
Av.	80.668	5.839	6.060	10.602	10.634
S.D.		0.009	0.007	0.002	0.003
	100.722	5.836	6.059	10.602	10.635
	100.845	5.835	6.056	10.603	10.638
	100.772	5.838	6.054	10.605	10.636
	101.081	5.824	6.046	10.598	10.634
	100.438	5.818	6.045	10.602	10.635
	100.420	5.842	6.065	10.603	10.635
Av.	100.713	5.832	6.054	10.602	10.636
S.D.		0.009	0.008	0.002	0.001

---

[a] Spectral bandpass.

[b] Standard deviation of a single determination.

5.7 Statistical Treatment of Experimental Apparent Specific Absorbances

The increase in the apparent specific absorbances of acidic potassium dichromate solutions with increasing concentration is clearly evident in the data shown in table 3. As discussed previously, this effect is due primarily to the concentration dependence of the $HCrO_4^-/Cr_2O_7^=$ equilibrium.

These data were subsequently analyzed and evaluated by H. H. Ku of the NBS Statistical Engineering Section. The average apparent specific absorbances obtained for the five concentration levels were fitted for each of the four wavelengths as a quadratic function and as a linear function of the average concentrations. The resulting residual standard deviations showed that the data fitted a linear function equally well as a quadratic function. While not expected, subsequent calculation of $HCrO_4^-/Cr_2O_7^=$ speciation using the dimerization equilibrium expression $K_D = [Cr_2O_7^=]/[HCrO_4^-]^2$ explained the reason for this observation. The relative amounts of $HCrO_4^-$ and $Cr_2O_7^=$ present for the 20-100 mg kg^{-1} range of $K_2Cr_2O_7$ concentrations employed are given in table 5. The value used for K_D [32.9 (mol kg^{-1})$^{-1}$] in this calculation was taken from an earlier publication [10]. The speciation data in table 5 clearly show that the percentage of chromium present in the dimer form ($Cr_2O_7^=$) is very nearly a linear function of the $K_2Cr_2O_7$ concentration over the five-fold concentration range used and hence explains the equal success of the linear fit.

Table 5. Composition of chromium (VI) solutions in 0.001N perchloric acid at 25 °C.

$K_2Cr_2O_7$ Conc., mg kg^{-1}	Percent Cr as $HCrO_4^-$	Percent Cr as $Cr_2O_7^=$
20	99.10	0.90
40	98.22	1.78
60	97.38	2.62
80	96.56	3.44
100	95.77	4.23

The results obtained from a linear fit of the apparent specific absorbances are shown in table 6. The uncertainties given were derived from estimates of all known systematic errors and the 95 percent confidence interval for the mean. A summary of the experimental conditions used in establishing the certified values of the apparent specific absorbances of SRM 935 and their uncertainties is given in table 7.

A similar statistical treatment of the average apparent specific absorbances obtained for the four wavelengths near the two isosbestic points (table 4) yielded the values given in table 8. These values are not certified because of their stronger dependence on wavelength accuracy and temperature control. They are given here to demonstrate that there are two isosbestic wavelengths in the weakly acidic potassium dichromate system which could be useful for verifying absorbance linearity.

Table 6. Certified values of the apparent specific absorbances, ε_a, of SRM 935 potassium dichromate in 0.001N perchloric acid at wavelengths 235, 257, 313 and 350 nm; T = 23.5 °C.

$K_2Cr_2O_7$ Conc., mg kg^{-1}	- - - - - - - - - ε_a, kg·g^{-1}·cm^{-1} - - - - - - - - -				
	235(1.2)[a]nm	257(0.8)nm	313(0.8)nm	350(0.8)nm	Uncertainty[b]
20[c]	12,260[d]	14.262	4.805	10.672	0.034
40	12,304	14.318	4.811	10.682	0.020[e]
60	12,347	14.374	4.816	10.692	0.020[e]
80	12,390	14.430	4.821	10.701	0.020[e]
100	12,434	14.486	4.827	10.711	0.020[e]

---

[a] Wavelength and, ( ), spectral bandpass.

[b] Includes estimated systematic errors and the 95 percent confidence interval for the mean.

[c] Nominal concentration.

[d] ε_a values are given to the third decimal place to preserve the smooth variation of the data with concentration, although the uncertainties are in the second decimal place.

[e] For wavelength of 313 nm, the uncertainty is reduced to half of these values for $K_2Cr_2O_7$ concentrations of 40, 60, 80 and 100 mg·kg^{-1}.

Table 7. Experimental conditions used in establishing the apparent specific absorbances, ε_a, of SRM 935.

Sample and Instrumental Parameters	Conditions
Weighing of $K_2Cr_2O_7$	Nominal 20 to 100 mg, ±0.004 mg
Weighing of $K_2Cr_2O_7$ solutions	Nominal 1000 g, ±0.020 g
Temperature	23.5 ± 0.2 °C
Curvette pathlength	Nominal 10 mm, ±0.0005 mm
Wavelength accuracy	±0.05 nm
Spectral bandpass	For λ = 235 nm, 1.2 nm; for all other wavelengths, 0.8 nm
Stray radiation	Less than 5 in 10^5 at 600 nm
Beam geometry	Convergent, f:10, corrected to normal incidence
Transmittance accuracy	1 part in 10^4

Table 8. Recommended values of the apparent specific absorbances, ε_a, of SRM 935 potassium dichromate in 0.001N perchloric acid at four wavelengths approximating the two isosbestic points; T = 23.5 °C.

$K_2Cr_2O_7$	ε_a, $kg \cdot g^{-1} \cdot cm^{-1}$			
Conc., mg kg^{-1}	322(0.8)[a]nm	323(0.8)nm	345(0.8)nm	346(0.8)nm
20[b]	5.854	6.074	10.604	10.626
40	5.849	6.069	10.603	10.628
60	5.844	6.064	10.602	10.631
80	5.838	6.059	10.601	10.633
100	5.833	6.054	10.600	10.636

---

[a] Wavelength and, ( ), spectral bandpass.
[b] Nominal concentration.

6. OTHER EXPERIMENTAL PARAMETERS AFFECTING THE APPARENT SPECIFIC ABSORBANCES OF SRM 935

6.1 Wavelength Accuracy

Except near the maxima and minima, the absorbances of acidic potassium dichromate solutions are strongly dependent on wavelength. Therefore, the accuracy of wavelength scale must be established before SRM 935 can be used as a standard to verify the accuracy of the absorbance scale.

In general, the wavelength accuracy of a spectrometer can be verified by the use of (a) emission sources capable of producing discrete radiations of suitable intensity and adequately spaced throughout the spectral range of interest and (b) solutions or glasses containing rare-earth elements. Since SRM 935 is being certified for use in the ultraviolet, the most useful and readily available source for checking wavelength accuracy in this region is the quartz low pressure mercury discharge tube. To calibrate the wavelength scale, this tube should be placed immediately in front of the entrance slit of the monochromator. Adequate eye protection against ultraviolet radiation should be worn while this discharge tube is being used.

A detailed description of the characteristic radiations and the use of low pressure discharge tubes for wavelength calibration is given in NBS Letter Circular LC-1017; "Standards for checking the Calibration of Spectrophotometers" by K. S. Gibson, H. J. Keegan, and J. C. Schleter, re-issued in January 1967. The data on the quartz low pressure mercury discharge, which are reproduced in table 9, were taken from this report.

Table 9. Characteristic radiations emitted by a quartz, low pressure, mercury discharge tube.

Wave-length (nm)	Note	Wave-length (nm)	Note	Wave-length (nm)	Note	Wave-length (nm)	Note
205.29		253.48	3	296.73		398.40	
222.47		253.65		302.15	6	---	
---		257.63		302.35		404.66	
---		260.32		302.56		407.78	
230.21		265.20	4	302.75		435.83	
232.32		265.37		312.57		491.60	
235.25		265.51		312.15		546.07	
237.83		269.95		313.18	7	576.96	9
239.94	1	275.28		334.15		579.07	
239.97		275.97		---		623.44	
244.69		280.35	5	365.01	8	671.64	
246.41		280.45		365.48		690.75	
248.20	2	284.78		366.29		1014.0	
248.27		289.36		366.33		1128.7	
248.38		292.54		390.64			

NOTES:

1. A value of 239.95 is recommended for the unresolved pair.
2. A value of 248.3 is recommended when the three lines are unresolved.
3. The intensity of 253.48 is negligible compared to that of 253.65. The latter value should be used when the lines are unresolved.
4. The 265.20 line is somewhat stronger than the others and a value of 265.3 is recommended when the three lines are unresolved.
5. These two lines are of approximately the same intensity and a value of 280.40 is recommended for the unresolved pair.
6. The two shorter lines are considerably stronger than the other two. It is probable that a value of 302.25 should be used for the unresolved lines.
7. A value of 313.16 is recommended for the unresolved pair.
8. With the arc used on the Beckman DU spectrophotometer the ratio of intensities for 365.01 : 365.48 : 366.29 is 100 : 48 : 36, approximately. The intensity of the 366.29 line appears negligible relative to that of 366.33.
9. These two lines are of approximately the same intensity and a value of 578.0 is recommended for the unresolved pair.

If additional information is needed, the user is referred to the NBS Letter Circular LC-1017 mentioned above or to the Manual on Recommended Practices in Spectrophotometry published by the American Society for Testing Materials, 1916 Race Street, Philadelphia, PA 19103. The wavelength scale should be accurate to ±0.2 nm in order for the SRM to be used properly.

The accuracy of the wavelength scale of the high-accuracy spectrometer used in this work was verified with a low pressure mercury discharge lamp using a slit of 0.1 mm which is equivalent to an effective spectral bandpass of 0.08 nm. The deviation of the wavelength scale from the true value in the range from 230 nm to 400 nm was found to be +0.05 nm and hence this value was applied to correct the scale.

6.2 Spectral Bandpass

Failure to select an appropriate spectral bandpass can lead to inaccurate results when optically non-neutral materials are used as transmittance or absorbance standards. In general, the degree of inaccuracy will depend on how sharp the absorbance peaks are. The use of too large a spectral bandpass will result in underestimating the absorbance at a maximum and overestimating the absorbance at a minimum.

As pointed out in the introduction of this publication, the use of acidic potassium dichromate as an absorbance standard is restricted to the verification of the transmittance/absorbance scale of spectrometers capable of providing spectral bandpasses of the magnitude indicated in table 10.

In a previous publication [10] a somewhat simplified mathematical treatment was presented on the effect of spectral bandpass on the measurement of absorbance at the wavelength maximum of a Gaussian peak. This study showed that the ratio of the spectral bandpass of the spectrometer to the natural bandwidth of the sample at half intensity of the fully resolved peak should not exceed 0.05 in order that the

Table 10. Variation of transmittance density with spectral bandpass of a 0.001N perchloric acid solution of potassium dichromate at wavelengths corresponding to absorbance maxima and minima.

Slitwidth, nm	Transmittance Density			
	235 nm Min.	257 nm Max.	313 nm Min.	350 nm Max.
0.2	0.2893 (0.24)[a]	0.3268 (0.34)	0.1293 (0.70)	0.2469 (1.1)
0.4	.2893 (0.48)	.3268 (0.68)	.1294 (1.4)	.2469 (2.1)
0.6	.2893 (0.72)	.3268 (1.0)	.1295 (2.1)	.2468 (3.2)
0.8	.2893 (0.96)	.3268 (1.4)	.1296 (2.8)	.2467 (4.2)
1.0	.2894 (1.2)	.3267 (1.7)	.1297 (3.5)	.2463 (5.3)
1.5	.2894 (1.8)	.3265 (2.6)	.1303 (5.3)	.2452 (8.0)
2.0	.2897 (2.4)	.3262 (3.4)	.1314 (7.0)	.2438 (10.6)
2.5	.2897 (3.0)	.3259 (4.3)	.1327 (8.8)	.2418 (13.3)
3.0	.2900 (3.6)	.3257 (5.1)	.1343 (10.5)	.2396 (15.9)

---

[a] Spectral bandpass, in nanometers, calculated from slitwidth and reciprocal dispersion data (supplied with the spectrometer).

measured absorbance be at least 0.999 of the true absorbance. A similar treatment of absorbance minima is not as straight forward because these result from two overlapping peaks. Based on these considerations and the data presented in table 10, the following restrictions are placed on spectral bandpass when SRM 935 is used as an absorbance standard: at λ=235 nm - 1.2 nm; at λ=257 nm - 1.4 nm; at λ=313 nm; and at λ=350 nm - 2.0 nm. For certification, a spectral bandpass of 1.2 nm was used at 235 nm, and 0.8 nm for the remaining wavelengths.

6.3 Stray Radiation

Stray radiation is defined as any radiation outside of the nominal spectral region isolated by the monochromator that reaches the detector. It is produced by scatter from the optics and walls of the monochromator and is present in varying amounts in all spectrometers. Although extensive literature [16,17,18] exists on the detection and measurement of stray radiation, no Standard Reference Materials are presently available for this purpose.

The spectrometrist must be aware of the potential presence of significant stray radiaton errors whenever absorbance measurements are made in a wavelength region where the spectrometer components are extended to their performance limit. These regions correspond to wavelengths where: (a) the source intensity is low, (b) the detector response in poor, and (c) the transmission of the monochromator is low. A detailed discussion of this instrumental parameter is given in ASTM Standard Method E 387-72. The procedures recommended in this work should be used to determine this parameter in the spectral range of interest. Measurements performed on the high-accuracy spectrometer have indicated that the stray radiation in this instrument is less than five parts in 10^5 at 600 nm.

NOTE: The user is cautioned that SRM 935 will provide meaningful data only when the three parameters discussed above—wavelength accuracy, adequate spectral bandpass and absence of stray radiation—are assured.

6.4 Beam Geometry

Since most commercial spectrophotometers use lens optics, the radiation impinging upon the sample usually is not collimated. This source of systematic error has been considered previously [10]. For angles of incidence up to five degrees, the absorbance correction is less than 0.1 percent. Insofar as the use of SRM 935 is concerned, this correction can be combined with the transmittance/absorbance scale correction.

6.5 Temperature

A change in temperature normally produces a change in the absorbance of an absorbing liquid. The sign and magnitude of the temperature coefficient are determined by the combined contributions of three effects. These are: (a) a change in concentration resulting from the thermal expansion or contraction of the solvent, (b) a change in ionization, protonation, or hydration of the absorbing species, and (c) a red or blue spectral shift resulting from either the addition or removal of thermal energy to the absorbing species. Generally, effects (a) and (c) are predominant and the sign of the temperature coefficient is *negative*, i.e., the absorbance decreases with increasing temperature. However, as seen previously [10], there are also systems that have positive temperature coefficients.

The variation of the absorbance of acidic potassium dichromate solution with temperature is the smallest that we have observed for any liquid filter that we have studied. For the four wavelengths selected for verifying the accuracy of the transmittance/absorbance scale — 235, 257, 313, and

350 nm — the specific absorbances decrease with increasing temperature. Over the range 20-30 °C, the temperature coefficients, k, are, respectively, -0.05, -0.05, -0.02, and -0.05 percent per °C. The temperature coefficients near the two isosbestic wavelengths are slightly larger, -0.06 and -0.08 percent per °C, respectively.

The apparent specific absorbances given in table 6 are certified at a temperature of 23.5 °C. However, SRM 935 can be used as an absorbance standard at any temperature in the range 20-30 °C provided corrections are made to the apparent specific absorbance data. The apparent specific absorbance at any temperature in this range can be calculated from the certified values and the appropriate temperature coefficient from the relation

$$\varepsilon_a^t = \varepsilon_a^{23.5}\left[1 + \frac{k}{100}(t - 23.5)\right] \qquad (4)$$

where:

ε_a^t = apparent specific absorbance at temperature t (°C)

$\varepsilon_a^{23.5}$ = specific absorbance certified at 23.5 °C

k = temperature coefficient, percent/°C.

For example, the apparent specific absorbance of a 20 mg kg^{-1} solution of SRM 935 in 0.001N perchloric acid at 30 °C and a wavelength of 350 nm can be calculated as follows: From table 6, the certified apparent specific absorbance for this concentration and wavelength is 10.672 kg·g^{-1}·cm^{-1}. Substituting this value into Eq. (4), the specific absorbance at 30 °C is:

$$\varepsilon_a^{30} = 10.672\left[1 - \frac{0.05}{100}(30 - 23.5)\right]$$

$$\varepsilon_a^{30} = 10.672\,[1 - 0.0033]$$

$$\varepsilon_a^{30} = 10.637 \text{ kg g}^{-1} \text{ cm}^{-1}.$$

6.6 Acidity

Acidic potassium dichromate solutions traditionally used in collaborative tests have been prepared in 0.01N sulfuric acid [1,2,3,4,5]. A number of studies, however, have confirmed the tendency of sulfate to form mixed complexes with chromium (VI) ions in acidic media [10,19,20,21]. Rather than risk making the $HCrO_4^-/Cr_2O_7^=$ system even more complex, the decision was made in an earlier study [10] that acidification with perchloric acid was preferable to acidification with sulfuric acid. For similar reasons it was decided that an acidity somewhat less than 0.01N was desirable in order to prevent partial formation of H_2CrO_4 from protonation of $HCrO_4^-$. At the same time, the acidity chosen could not be too low because $HCrO_4^-$ would partially ionize to give $CrO_4^=$, a chromium (VI) species which has quite different absorption characteristics. As a practical compromise for minimizing the protonation and ionization processes, an acidity of 0.001N (pH = 3) was finally selected. At this acidity, less than 0.1 percent of the total chromium in the absorbing system is present in either the H_2CrO_4 or $CrO_4^=$ form. Close control of acidity at the 0.001N level is not critical in the use of SRM 935 as an absorbance standard. The data in tables 6 and 8 are equally valid for perchloric acid solutions whose pH is in the range 2.5 to 3.5.

6.7 Water Purity

Since SRM 935 will be issued in solid form, attention must be given by the user to the quality of the distilled water used to prepare the standard solutions. Since acidic potassium dichromate is a potential oxidant, the water must be checked and shown to be free of reducing impurities in order that the SRM provide accurate information. A simple but definitive test is illustrated in figure 4. In this test a 500-fold dilution of a 100 mg kg^{-1} solution of

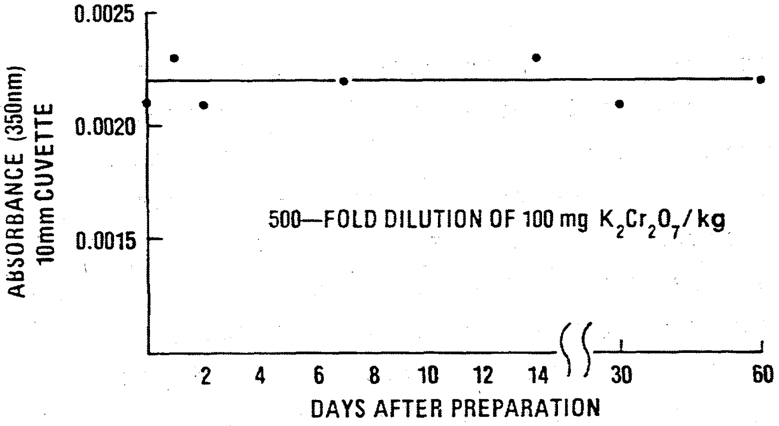


Figure 4. Test of the distilled water for reducing impurities.

$K_2Cr_2O_7$ (A = 1.0) was made with the distilled water to be tested and its absorbance measured at 350 nm. The measured absorbance (A = 0.002) agreed with the expected value, thus indicating that no reduction of chromium (VI) occurred. Subsequent measurements showed that this solution was stable for at least two months. Hence, any solution standards prepared with this water should be stable for the same period provided they are adequately protected against evaporation losses and exposure to light.

All of the measurements described in this publication were made using distilled water provided to the Analytical Chemistry Division from a central supply. The still, condenser system, storage tanks and the copper pipes supplying this distilled water to the individual laboratories are all tin-lined. Although this water is not free of organic compounds, the test described above shows that these compounds do not reduce chromium (VI) under the acidity conditions used. In this regard, it is interesting to note that the oxidation potential of potassium dichromate is strongly acid dependent. By preparing the $K_2Cr_2O_7$ solutions in 0.001N rather than 0.01N perchloric, the oxidation potential of the chromium (IV) system is lowered by about 13 percent. Whenever the quality of the distilled water is suspect, it should be redistilled from an alkaline potassium permanganate solution in order to preoxidize any reducing impurities.

7. INSTRUCTIONS FOR USING SRM 935 AS AN ABSORBANCE STANDARD

The procedure recommended for using SRM 935 to verify the accuracy of the absorbance scales of narrow bandpass absorption spectrometers is identical to the one used in the certification of this Standard Reference Material. All the operations involved in this certification were described in detail in Section 5 of this publication. Once the solutions of SRM 935 have been prepared accurately, their absorbances are measured on the spectrometer to be verified using the instrumental conditions and restrictions described in Sections 5 and 6. Strict attention must be given to wavelength accuracy, adequate spectral bandpass, absence of stray light, and temperature.

The certification of SRM 935 for apparent specific absorbances provides considerable flexibility in the use of this material for verifying the accuracy of the absorbance scale. The user for example, is not required to prepare specified concentrations of $K_2Cr_2O_7$ solution. The only requirement is that the concentration of every solution for checking the scale be known accurately. Although the certified values given in table 6 are for nominal $K_2Cr_2O_7$ concentrations of 20, 40, 60, 80 and 100 mg kg^{-1} the appropriate specific absorbances for other concentrations can be obtained by linear interpolation.

The derivation of ε_a by linear interpolation and its subsequent use to calculate an accurate value of A_a are described below, assuming the following experimental conditions:

$\lambda = 350.0 \pm 0.2$ nm; spectral bandpass ≤ 0.8 nm
$b = 0.9982$ cm
$c = 0.04375$ g kg^{-1}
$t = 23.5$ °C

From table 6, the ε_a for $\lambda = 350$ nm and concentrations of 0.040 and 0.060 g kg^{-1} are 10.682 and 10.692, respectively. The ε_a for $c = 0.04375$ g kg^{-1} is:

$$\varepsilon_a = 10.682 + \frac{0.04375 - 0.040}{0.060 - 0.040}(10.692 - 10.682)$$

$$\varepsilon_a = 10.682 + 0.0019 \tag{6}$$

$$\varepsilon_a = 10.684$$

The corresponding apparent absorbance A_a is given by:

$$A_a = \varepsilon_a \cdot b \cdot c \tag{7}$$

$$A_a = 10.684 \times 0.9982 \times 0.04375$$

$$A_a = 0.4666$$

The uncertainty ΔA_a in the calculated A_a is determined from the combined uncertainties in ε_a, b, and c in equation 7, provided no other systematic errors are present. Thus, using the propagation of error formulas given by Ku [22]

$$\Delta A_a = bc|\Delta\varepsilon_a| + \varepsilon_a c|\Delta b| + \varepsilon_a b|\Delta c| \tag{8}$$

To evaluate ΔA_a, the appropriate value of $\Delta\varepsilon_a$ is taken from table 6 and the Δb and Δc values must be provided by the user. For the example taken, solution of equation 8 for the $\Delta\varepsilon_a$ term gives

$$\Delta A_a = 1 \times 0.044(0.020) + \varepsilon_a c|\Delta b| + \varepsilon_a b|\Delta c| \tag{9}$$

$$\Delta A_a = \pm\{0.00088 + \varepsilon_a c|\Delta b| + \varepsilon_a b|\Delta c|\} \qquad (10)$$

In the experiments performed to obtain the certified ε_a values in table 6, our uncertainties for b and c did not exceed 1 part in 10^4 and 2 parts in 10^4, respectively. Under these conditions, the combined contribution of Δb and Δc to ΔA_a does not exceed 0.0002 and $\Delta A_a = \pm 0.0010$.

In practice, the user may not know b and c as accurately as in the above example. Solution of equation (10) for the case in which b and c are known to 1 part in 10^3 gives

$$\Delta A_a = 0.00088 + 10.7(0.044)(0.001) + 10.7(1)(4.4 \times 10^{-5})$$

$$\Delta A_a = 0.00088 + 0.00047 + 0.00047$$

$$\Delta A_a = \pm 0.0018$$

The calculations of ε_a, A_a and ΔA_a for other concentrations, wavelengths, and uncertainties in b and c are performed in a similar fashion.

The use of SRM 935 to verify the accuracy of the absorbance scale of a precision transmission spectrometer is illustrated in figure 5. These measurements were made using a series of five $K_2Cr_2O_7$ solutions having nominal concentrations of 20, 40, 60, 80 and 100 mg kg^{-1}. In each instance the measured absorbance is higher than the certified values and should be corrected by subtracting the appropriate value of A_a(Meas.) - A_a(Calc.).

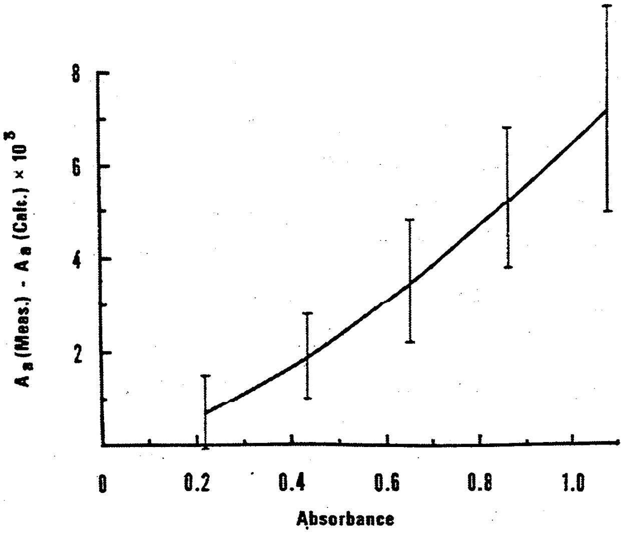


Figure 5. Correction curve for the absorbance scale of a precision spectrometer. The error bars are the sum of the errors arising from the uncertainties in the certified apparent specific absorbances ε_a, cuvette pathlength b, and concentration c.

Although solutions of this SRM should be prepared strictly on a weight/weight basis, there are undoubtedly users who will desire to prepare and express concentrations on a weight/volume basis. While this approach is not recommended, the certified apparent specific absorbances in table 6 can be recalculated for the case in which the $K_2Cr_2O_7$ concentration is expressed in $g \cdot L^{-1}$. This recalculation simply requires dividing the certified values by the appropriate density given in table 11. The uncertainties and temperature coefficients remain unchanged. Anyone wishing to use this approach should calibrate the volumetric flasks used. NBS recommendations for performing this calibration are given in two papers included in the Appendix.

Table 11. Densities of 0.001N perchloric acid solutions of potassium dichromate at 23.5 °C.

$K_2Cr_2O_7$ Conc., $mg \cdot kg^{-1}$	Density, $kg \cdot L^{-1}$
20	0.99746
40	0.99747
60	0.99749
80	0.99750
100	0.99752

8. USE OF SRM 935 TO VERIFY ABSORBANCE LINEARITY

The apparent specific absorbances of 0.001N perchloric acid solutions of SRM 935 at wavelengths near the two isosbestic points in the $HCrO_4^-/Cr_2O_7^=$ spectra (figure 1) have been presented in table 7. Inspection of these data show that, of the four wavelengths selected, the calculated values for 345 nm are, within an uncertainty of 0.1 percent, independent of concentration over the range of 20-100 $mg \cdot kg^{-1}$ $K_2Cr_2O_7$. This relation was not expected since none of the data in table 7 have been corrected for internal multiple reflection effects. The constancy of the uncorrected specific absorbances at 345 nm therefore must result from the presence of compensating effects.

The most plausible explanation for the observed invariance in the apparent specific absorbance data at 345 nm is that the internal reflection corrections, which are proportionally larger at lower absorbances [15], are almost exactly offset by the effect of the variable $HCrO_4^-/Cr_2O_7^=$ speciation at the higher absorbances. Following this reasoning, the $Cr_2O_7^=$ ion must have a slightly larger specific molar absorbance at 345 nm than the $HCrO_4^-$ ion. Consequently the true isosbestic point is at a slightly shorter wavelength than those used for measurement. A similar consideration of the data reported for 322 and 323 nm indicate that this isosbestic point is also slightly below the wavelengths chosen.

For the purpose at hand the important fact remains that the apparent specific absorbances of 0.001N perchloric acid solutions of $K_2Cr_2O_7$ at 345 nm are independent of concentration at the one part in a thousand level over the range 20-100 $mg \cdot kg^{-1}$. Consequently, if the absorbance scale of a narrow bandpass ($\leq$2 nm) spectrometer is linear, the apparent absorbances of a series of $K_2Cr_2O_7$ solutions at this wavelength should be a linear function of concentration. Such a test may be particularly useful to the clinical chemists who make a proportionally large number of measurements in the wavelength range of 340-350 nm.

In summary, an outline of the step-by-step operations required to use SRM 935 for verification of a spectrometer is given below.

- Weigh 20, 40, 60, 80 and 100 mg of SRM 935 to ±0.02 mg and transfer quantitatively to five 1-liter borosilicate volumetric flasks that have been preweighed, with their individual Teflon stopper, to ±0.01 g.
- Dissolve each sample in 200 to 300 mL of distilled water that is free of reducing impurities, add 1 mL of 1N perchloric acid, and dilute approximately to volume with distilled water.
- Stopper, mix, and weigh each flask and contents to ±0.01 g.
- Prepare a blank solution by diluting 1 mL of 1N perchloric acid to 1 liter with the same distilled water.

- Place a clean, calibrated, fused-silica (non-fluorescent) cuvette in the temperature-controlled sample compartment of the spectrometer to be verified. The temperature should be controlled to ±0.2 °C and in the range of 20 to 30 °C.
- Using a borosilicate, Pasteur-type pipette, rinse the cuvette with six volumes of the blank solution. Transfer a seventh volume and close the cuvette with a Teflon stopper.
- After 10 minutes measure the apparent absorbance of the blank solution against air at wavelengths 235, 257, 313 and 350 nm, using the spectral bandpasses given in table 6. For verification of linearity, measure the apparent absorbance also at 345 nm. The data obtained represent the values of D_B in equation (3) on page 18.
- In a similar manner perform apparent absorbance measurements on the five solutions of SRM 935 potassium dichromate. For each concentration, rinse the cuvette a minimum of six times before beginning measurements. The data represent the values of D_S in equation (3) on page 18.
- Use the values of D_S and D_B thus obtained to calculate the measured A_a of each of the five concentrations.
- Compare the experimental values of A_a for these five concentrations with the accurate A_a calculated from the certified values of apparent specific absorbances, ϵ_a, given in tables 6 and 7 of this publication and comprising table 1 of the certificate (a copy of the certificate is included in the Appendix).

- The differences between the calculated and measured A_a values are used to correct the absorbance scale of the spectrometer as illustrated in figure 5. The correction curves for the four wavelengths at which ε_a is certified should be similar.

It must be added, however, that the absorbance linearity test described above should not be associated with the accuracy of the transmittance/absorbance scale of the spectrometer being tested. Detailed discussions of important distinctions between ascertaining transmission accuracy and linearity will be found in references 23 and 24.

9. SUMMARIZING REMARKS

Potassium dichromate is the first SRM to be issued by the NBS Office of Standard Reference Materials for use as an ultraviolet absorbance standard. The fact that this compound has been widely used for many years in the collaborative testing of spectrometers suggests that it should play a more significant role in the future since, for the first time, the absorbance measurements have been performed with an instrument of proven accuracy. The certification of the apparent specific absorbances of solutions prepared from this SRM over a range of concentrations should give the analyst the flexibility necessary to verify the accuracy of any portion of the absorbance scale that may be desired. In addition the capability of using the isosbestic data as a verification of absorbance linearity suggests a number of other useful applications.

In conclusion, it is believed that SRM 935, when used in conjunction with a calibrated quartz cuvette (SRM 932), will provide a valid means to verify the accuracy of the absorbance scale of narrow bandpass spectrophotometers in the ultraviolet over the wavelength range of 235-350 nm.

10. ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of the following members of the NBS staff:

- K. D. Mielenz, Research Physicist, Analytical Chemistry Division, for many helpful discussions of fundamental spectrophotometric parameters and for critically reading the manuscript.
- G. Marinenko, Research Chemist, Analytical Chemistry Divison, for performing the coulometric assays.
- J. A. Norris, Research Chemist, Analytical Chemistry Divison, for performing the emission spectrographic analyses.
- H. H. Ku, Senior Statistician, Statistical Engineering Section, for the evaluation and statistical treatment of the experimental data.
- R. W. Seward, Technical Representative, Office of Standard Reference Materials, for obtaining and providing the lot of potassium dichromate used for certification.
- I. L. Barnes and J. R. DeVoe, Section Chiefs, Analytical Chemistry Division, for their kind support and encouragement throughout this work.

The authors also wish to acknowledge the cooperation and early financial support of the National Institute of General Medical Sciences in the research leading to this SRM. The enthusiastic encouragement of George N. Bowers, Jr., M.D., Hartford Hospital, Hartford, Connecticut and Royden N. Rand, Ph.D., Eastman Kodak Co., Research Laboratories, Rochester, New York is likewise gratefully acknowledged.

11. REFERENCES

[1] Gridgemen, N. T., Photoelectric Spectrometry Group Bull. 4, 67-79 (1951).

[2] Photoelectric Spectrometry Group Bull. 16, 441-457 (1965).

[3] Ketelaar, J. A. A., Fahrenfort, J., Hass, C., and Brinkman, G. A., Photoelectric Spectrometry Group Bull. 8, 176-179 (1955).

[4] Vandenbelt, J. M., and Spurlock, C. H., J. Opt. Soc. Am. 45, 967-970 (1955).

[5] Vandenbelt, J. M., J. Opt. Soc. Am. 50, 24-27 (1960).

[6] Mavrodineanu, R. in NBS Technical Note 544, 6-16 (July 1969—June 1970; and 584, 2-21 (July 1970—June 1971).

[7] Mavrodineanu, R., J. Res. Nat. Bur. Stand. (U.S.) 76A (Phys. and Chem.), No. 5, 405-425 (Sept.-Oct. 1972).

[8] Mavrodineanu, R., J. Res. Nat. Bur. Stand. (U.S.) 80A (Phys. and Chem.), No. 4, 637-641 (July-Aug. 1976).

[9] Burke, R. W., and Mavrodineanu, R., J. Res. Nat. Bur. Stand. (U.S.) 80A (Phys. and Chem.), No. 4, 631-636 (July-Aug. 1976), included in the Appendix.

[10] Burke, R. W., Deardorff, E. R., and Menis, O., J. Res. Nat. Bur. Stand. (U.S.) 76A (Phys. and Chem.), No. 5, 469-482 (Sept.-Oct. 1972), included in the Appendix. See also NBS Technical Note 544, 17-34 (July 1967—June 1970; and 584, 21-45 (July 1970—June 1971).

[11] Yoshimori, T. and Sakaguchi, N., Talanta 22, 233-238 (1975).

[12] Mavrodineanu, R. and Lazar, J. W., Nat. Bur. Stand. (U.S.), Spec. Publ. 260-32, 26 pages (Dec. 1973), and in Clin. Chem. 19, 1053-1057 (1973), included in the Appendix.

[13] McCormick, P. G., Burke, R. W., and Doumas, B. T., Clin. Chem. 18, 854-856 (1972).

[14] Mielenz, K. D. and Mavrodineanu, R., J. Res. Nat. Bur. Stand. (U.S.) 77A, (Phys. and Chem.), No. 6, 699-703 (Nov.-Dec. 1973).

[15] Burnett, R. W., Anal. Chem. 45, 383-385 (1973).

[16] Perry, J. W., Photoelectric Spectrometry Group Bull. 3, 40 (1950).

[17] Slavin, W., Anal. Chem. 35, 561-566 (1963).

[18] Paulson, R. E., Appl. Opt. 3, 99-104 (1964).

[19] Tong, J. and King, E. L., J. Am. Chem. Soc. 75, 6180-6186 (1953).

[20] Davies, W. G., and Prue, J. E., Trans. Faraday Soc. 51, 1045-1051 (1955).

[21] Haight, G. P., Richardson, D. C., and Coburn, N. H., J. Inorg. Chem. 3, 1777-1780 (1964).

[22] Ku, H. H., J. Res. Nat. Bur. Stand. (U.S.), 70C, (Eng. and Instr.), No. 4, 263-273 (Oct.-Dec. 1966).

[23] Reule, A. G., Appl. Optics 7, 1023-1028 (1968) and J. Res. Nat. Bur. Stand. (U.S.) 80A, No. 4, 609-624 (1976). See also NBS Technical Note 544, 17-34 (July 1967—June 1970); and 584, 21-45 (July 1970—June 1971).

[24] Sloane, H. J. and Gallaway, W. S., Appl. Spectry. 31, 25-30 (1977).

12. APPENDICES

For additional information and as a convenience to the reader, reprints of papers which are considered particularly relevant to the certification and use of SRM 935 have been included in the Appendix of this publication together with a copy of the certificate. These are:

1. Comments on Spectrometry Nomenclature.
2. Liquid Absorbance Standards.
3. Standard Reference Materials: Standard Quartz Cuvettes for High–Accuracy Spectrophotometry.
4. Acidic Potassium Dichromate Solutions as Ultraviolet Absorbance Standards.
5. Testing of Glass Volumetric Apparatus.
6. The Calibration of Small Volumetric Laboratory Glassware.
7. Certificate of Analysis, SRM 935, Crystalline Potassium Dichromate for Use as an Ultraviolet Absorbance Standard.

APPENDIX I

Reprinted from **ANALYTICAL CHEMISTRY**, Vol. 48, Page 1093, June 1976

Comments on Spectrometry Nomenclature

Sir: As an optical physicist who collaborates with analytical chemists in the development and use of absorption and luminescence spectrometers, I have become increasingly aware of the fact that our vocabularies are not always the same. Although some of these differences have been pointed out before (*1*) it seems worthwhile to familiarize the readers of this journal with the currently prevailing terminology of physical optics. Perhaps the following comments may be helpful to editors, authors, and nomenclature committees in establishing a common and consistent terminology that can be used in all branches of spectrometry and would save readers a good deal of puzzlement and reading between lines. The main purpose of these comments is to emphasize the need for interdisciplinary efforts in defining spectrometry nomenclature without perpetuating the inconsistencies that exist at present. They do not represent an official position of the National Bureau of Standards.

The basic concept of modern optical terminology (*2, 3*) is to combine nouns and adjectives in order to describe quantities and properties as explicitly as necessary, rather than using glossaries of short names for them. This "Chinese Restaurant" method of nomenclature (*4*) offers flexibility in introducing new terms, and has recently been extended to include the photon quantities used in photochemistry and similar disciplines dealing with the interaction of light and matter (*5*).

The basic list of nouns describing the transport of energy according to the laws of geometrical optics is:

Energy, Q.
Energy density, $u = \mathrm{d}Q/\mathrm{d}V$. Energy per unit volume.
Flux, $\Phi = \mathrm{d}Q/\mathrm{d}t$. Time rate of energy flow.
Flux density, $W = \mathrm{d}\Phi/\mathrm{d}A$. Flux per unit area.
Intensity, $I = \mathrm{d}\Phi/\mathrm{d}\Omega$. Flux per unit solid angle.
Sterance, $L = \mathrm{d}^2\Phi/\mathrm{d}(A\cos\Theta)\mathrm{d}\Omega$. Flux per unit projected area and unit solid angle.

(A number of alternative and additional terms have been proposed. For example: *pointance* to replace the continually misused term *intensity; incidance* and *exitance*, or *incident areance* and (*transmitted, emitted*, etc.) *areance* for the flux densities arriving at and leaving a surface; *sterisent* for the sterance generated per unit path length by emission or scattering into the direction of propagation; and *fluence* for the surface energy density incident upon a volume element irradiated from within a large solid angle of rays (F. E. Nicodemus, private communication). As these have not yet been accepted generally, they were not included here. However, the new term *sterance* was included as it avoids the misnomer *photon radiance* that has appeared in papers on luminescence spectrometry.)

These nouns are modified by the adjective *radiant*, and the above symbols are written with a subscript 'e' (for energy), when radiometric units are used. The modifiers *luminous* and *photon* are used in conjunction with subscripts 'v' (for visual) and 'p' (for photon) to indicate the use of photometric and photon units, respectively. For example:

Radiant energy density, u_e[J m^{-3}].
Luminous intensity, I_v[lm sr^{-1}].
Photon sterance, L_p[E m^{-2} sr^{-1} s^{-1}].

(The basic unit for photon energy used here is the *einstein* [E], defined as one mole of photons. It is not an SI unit, but is used so extensively in photochemistry and photobiology that its acceptance as a supplementary SI unit may be desirable.)

The additional modifier *spectral* and subscripts λ and σ are used to denote derivatives of radiometric and photon quantities with respect to wavelength and wavenumber, respectively. Thus:

Spectral radiant energy density, $u_{e,\lambda} = \mathrm{d}u_e/\mathrm{d}\lambda$ [J m^{-4}].
Radiant energy density per unit wavelength interval.
Spectral photon intensity, $I_{p,\sigma} = \mathrm{d}I_p/\mathrm{d}\sigma$ [E sr^{-1} s^{-1} m].
Photon intensity per unit wavenumber interval.

Any of these can be modified further; such as: *fluorescence photon flux, transmitted spectral radiant flux density*, or *absorbed luminous energy*. Usually, it is possible to drop most of the modifying adjectives as well as the subscripts e, v, or p, once the context has been clearly established or whenever a distinction is not necessary.

The same method of nomenclature also provides a simple and logical way of specifying the precise meaning of the quantities and material properties commonly measured in analytical spectrometry. Thus *radiant absorptance* α_e and *photon absorptance* α_p should be used for the ratios of the radiant or photon fluxes absorbed by a sample to those incident upon it, when measured with a large bandwidth so that these two ratios are not the same. On the other hand, *spectral absorptance* $\alpha(\lambda)$ is sufficiently accurate in the case of measurements made with a small bandwidth. Similarly, luminescence yields (the ratios of the radiant or photon fluxes emitted by a sample to those absorbed by it) should be designated as *radiant yield* η_e or *photon yield* η_p (not *energy yield* or *quantum yield*). In this case, *spectral radiant yield*, $\eta_{e,\sigma} = \mathrm{d}\eta_e/\mathrm{d}\sigma$, and *spectral photon yield*, $\eta_{p,\sigma} = \mathrm{d}\eta_p/\mathrm{d}\sigma$, are different quantities even in the limit of infinitely narrow bandwidths, and thus should be referred to by these names.

[The dependence on wavelength or wavenumber is indicated by a subscript ($\eta_{p,\sigma}$) when the spectral distribution is defined as a derivative, but in functional form [$\alpha(\lambda)$] in the case of spectral distributions that are not derivatives. This is an important distinction that must also be borne in mind in the presentation of spectra. The positions of the peaks and valleys in derivative spectra depend on the units used, so that these should not be published in the form of a single graph with dual scales (such as wavelength and wavenumber, or spectral radiant and photon yield).]

In addition to this general description of current optical terminology, the following comments are made in direct reference to the nomenclature list in the December 1975 issue of *Analytical Chemistry* (*6*).

1) This list cautions not to use *optical density* instead of *absorbance*, but defines the latter as "the logarithm to the base ten of the reciprocal of transmittance"—which is the definition of optical density. *Internal transmittance* should have been substituted for *transmittance* in this def-

inition of absorbance. The International Lighting Vocabulary suggests *transmission density* and *internal transmission density* instead of optical density and absorbance. This is more determinative, and also fits into a general scheme where *reflection density* is defined as the negative logarithm to the base ten of reflectance. On the other hand, transmission and reflection density are operational quantities that merely express measured data on a logarithmic scale, whereas absorbance is directly related to molecular constants through the Lambert–Beer and Strickler–Berg equations. Thus, in my opinion, it should be retained as a separate term. I believe that all confusion would be avoided by defining:

Transmittance, τ. Ratio of the flux transmitted by a sample to the flux incident upon it.

Internal transmittance, τ_i. Transmittance exclusive of losses at boundary surfaces and effects of interreflections between them.

Transmittance density, $D = -\log_{10}\tau$. Negative logarithm to base ten of transmittance.

Absorbance (Internal transmission density), $A = -\log_{10}\tau_i$. Negative logarithm to base ten of internal transmittance.

2) The definitions of *absorptivity* in chemistry and physics are not the same. In chemistry, it means absor*b*ance per unit path length and unit concentration (A/bc), whereas the International Lighting Vocabulary (2) defines it as internal absor*pt*ance per unit path length ($\mathrm{d}\alpha_i/\mathrm{d}b$). Similarly, it specifies *transmissivity* as internal transmittance per unit path length and *reflectivity* as the reflectance of a thick layer (so that a further increase in thickness will no longer change its value). Generally, terms ending in *-ance* represent sample properties, whereas terms ending in *-ivity* denote material properties that are independent of sample geometry. The quantity A/bc does not fall in this latter category as it is also independent of sample concentration. The International Union for Pure and Applied Chemistry, apparently aware of this discrepancy, has suggested calling it *absorption coefficient*. However, as this name has been given different and mutually inconsistent meanings in the past, a less ambiguous word—perhaps *specific absorbance*—would have been better. Therefore it is proposed to define:

Absorptivity, $a = \mathrm{d}\alpha_i/\mathrm{d}b$ [m^{-1}]. Internal absorptance per unit path length.

Specific absorbance, $\epsilon = A/bc$ [$\mathrm{kg}^{-1}\ \mathrm{m}^2$]. Absorbance per unit path length and unit concentration.

Specific molar absorbance, $\epsilon_m = A/bc_m$ [$\mathrm{mol}^{-1}\ \mathrm{m}^2$]. Absorbance per unit path length and unit molar concentration.

3) The December 1975 nomenclature list also implies that the Beer–Lambert laws are the same. The correct definitions are:

Beer's law: Absorbance is proportional to concentration.

Lambert's law: Absorbance is proportional to path length. Also called *Bouguer's law.*

4) The definition of the *ångström* unit in terms of the red line of cadmium has been abrogated several years ago. It is now defined as 10^{-10} m, exactly. However, the ångström is not an SI unit, and has been sanctioned by the International Committee on Weights and Measures only as a supplementary unit that will eventually be abandoned (7). Therefore, authors should be encouraged to use *micrometers* or *nanometers.*

5) The December 1975 list defines *spectrometry* as the "measurement of spectra", but restricts the meaning of *spectrometer* to "instrument with an entrance slit, a dispersing device, and with one or more exit slits . . .". As this excludes non-dispersive and slit-less instruments, such as Fourier and Girard spectrometers, it would be more consistent to call any instrument used for spectrometry a *spectrometer.* Therefore:

Spectrometer: Instrument for the measurement of spectra.

The general term for instruments that measure spectral distributions of radiometric quantities is *spectroradiometer.* However, as this implies measurements in absolute units, *spectrometer* is a better term for the simpler, usually ratio-forming instruments used in analytical spectrometry. Thus, a *spectrophotometer* could also be called *absorption spectrometer*, and *fluoroescence spectrometer* would end the controversy of *spectrofluorimeter* vs. *spectrofluorometer.*

(Strictly speaking, the word *spectrophotometer* is a misnomer. A *photometer* is an instrument that measures luminous flux in lumens. Since the adjective *luminous* implies the integral effect of visual radiation as perceived by the human eye, the spectral analysis of luminous flux has no physical meaning. However, in view of the firmly established meaning of *spectrophotometer*, it is not suggested to change it, although a scanning of the recent literature shows an increasing usage of *absorption spectrometer*.)

LITERATURE CITED

(1) G. A. W. Rutgers, in "Accuracy in Spectrophotometry and Luminescence Measurements," *Nat. Bur. Stand. (U.S.), Spec. Publ.* **378**, 53 (1973).

(2) "International Lighting Vocabulary", 3rd ed., Publication CIE No. 17, Paris, 1970.

(3) Editor's Page, *J. Opt. Soc. Am.*, **57**, 854 (1967).

(4) J. Geist and E. Zalewski, *Appl. Opt.*, **12**, 435 (1973).

(5) J. J. Murray, F. E. Nicodemus, and I. Wunderman, *Appl. Opt.*, **10**, 1465 (1971).

(6) "Spectrometry Nomenclature", *Anal. Chem.*, **47**, 2526 (1975).

(7) "The International System of Units (SI)", *Nat. Bur. Stand. (U.S.), Spec. Publ.* 330 (1971).

Klaus D. Mielenz

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

RECEIVED for review September 26, 1975. Accepted December 23, 1975.

APPENDIX II

JOURNAL OF RESEARCH of the National Bureau of Standards—A. Physics and Chemistry
Vol. 76A, No. 5, September–October 1972

Liquid Absorbance Standards

R. W. Burke, E. R. Deardorff, and O. Menis

Institute for Materials Research, National Bureau of Standards, Washington, D.C. 20234

(June 14, 1972)

Errors in the measurement of the absorbances of liquid filters result from instrumental and chemical uncertainties. This paper presents a systematic study of these variables on the absorbances of selected filters. Three types of liquid filters are discussed. These are (1) individual solutions of high purity compounds, (2) composite mixtures and (3) aqueous solutions of organic dyes. The accuracy of the absorptivity data is established using NBS-calibrated glass filters. The magnitude of the errors arising from spectral bandpass, beam geometry, stray light, internal multiple reflections, and refractive index are delineated. Finally, as a practical outgrowth of this study, the development and issuance of NBS Standard Reference Material 931, Liquid Absorbance Standards for Ultraviolet and Visible Spectrophotometry, is described.

Key words: Absorptivity data; accuracy; liquid absorbance standards; Standard Reference Materials.

I. Introduction

In the use of filters for checking the accuracy of the photometric scale of spectrophotometers, one needs materials which exhibit absolute spectral neutrality. Such ideal filters are not available at present. The use of the materials which have been suggested is limited by instrumental and sample variables. For this reason, one must carefully specify conditions which define the other sources of errors.

The first part of this paper reviews several of the more important instrumental parameters and discusses their effects on absorbance measurements. Examples from the literature are given to illustrate the magnitude of the errors arising from spectral bandpass, stray light, nonparallel radiation, and multiple reflections. The second part presents some experimental studies of several materials which may serve as calibration "filters" or standards.

II. Instrumental Parameters

A. Wavelength Accuracy

The absorbances of most samples are sufficiently wavelength dependent that even in the most favorable regions of maxima and minima a wavelength error of 1–2 nm can produce absorbance errors of a few tenths of a percent. When performing measurements on the slopes of absorption peaks, the wavelength setting is obviously much more critical and errors are typically several percent per nanometer. Wavelength accuracy, therefore, becomes especially important when making absorbance measurements at, for example, an isosbestic point.

A line source provides the most definitive means f establishing the accuracy of the wavelength scal The best single source is a mercury lamp which ma be used throughout the range of 200–1000 nm. F calibration in the visible region, helium lines ar also useful.

Gibson [1][1] has discussed the use of mercury an helium sources and has listed those lines best suite for wavelength calibration. Other sources which hav been used include neon, cadmium, cesium, and sodiu The M.I.T. Wavelength Tables [2] summarizes the mo prominent emission lines of these elements. Not a wavelengths are given and care must be taken i using any of these lines in order not to confuse closel adjacent lines with the one being checked. For thos spectrophotometers having a hydrogen or deuteriu source, the emission lines at 486.13 and 656.28 n (H) or 485.99 and 656.10 nm (D) may provide co venient checks at these wavelengths.

Calibrated holmium oxide and didymium glasses ma be useful secondary standards, particularly for chec ing recording spectrophotometers in which a dynam check of the instrument is often desirable. Th apparent absorption maxima of these filters, howeve may vary with spectral bandpass. Therefore, for th highest accuracy, they should be calibrated for th spectral bandpass at which they are to be used.

In general, prism instruments require more freque calibration than the grating type because their di persion is temperature dependent. Corrections in th visible region are of the order of 0.1 nm per degre Celsius. Hysteresis effects must also be considere

---

[1] Figures in brackets indicate the literature references at the end of this paper.

and the wavelength settings should always be approached from the same direction.

B. Finite Bandwidth

Spectral bandpass differences undoubtedly account for a significant portion of the discrepancies in molar absorptivity values reported in the literature. Some have resulted from the employment of inadequate instrumentation; others have resulted from improper use of these instruments or complications arising from solution equilibria.

Increasing the spectral bandpass at an absorption maximum has two effects on apparent peak heights: (1) the observed values are always less than the true values and (2) the differences between the two are proportionally greater at higher absorbances. Thus, for a finite bandpass, a plot of absorbance versus concentration or path length will always have a smaller slope than it does in monochromatic radiation and, in addition, will be concave to the concentration axis. Figure 1 illustrates these effects. The reverse behavior will be observed for measurements at an absorption minimum. Meehan [3] has given a simple example which verifies these two effects mathematically.

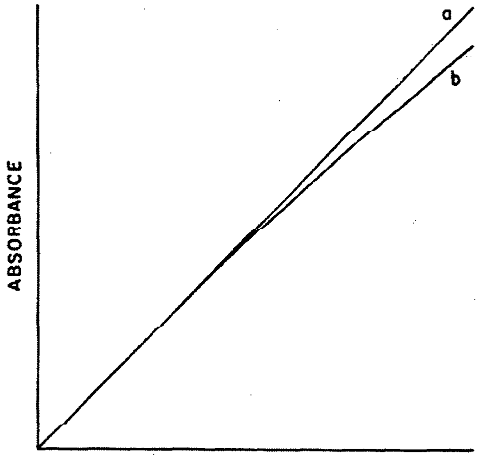


FIGURE 1. Effect of spectral bandpass on absorbance: (a) Monochromatic radiation (b) Finite bandwidth.

Consider that an absorbing system is illuminated by two monochromatic radiations of wavelengths λ_1 and λ_2 and that the Lambert-Beer law is obeyed at each wavelength with absorptivities a_1 and a_2. If the effective intensities of the two wavelengths are I_1 and I_2, respectively, the transmittance, T, is

$$T = (I_1 \cdot 10^{-a_1bc} + I_2 \cdot 10^{-a_2bc})/(I_1 + I_2) \qquad (1)$$

where b = path length and c = concentration. If λ_1 is the wavelength of maximum absorption so that $a_1 > a_2$, then

$$T = \frac{1}{(1+r)} \cdot 10^{-a_1bc}[1 + r \cdot 10^{(a_1-a_2)bc}] \qquad (2)$$

where $r = I_2/I_1$. The absorbance, A, defined as $-\log T$ is

$$A = a_1bc + \log(1+r) - \log[1 + r \cdot 10^{(a_1-a_2)bc}] \qquad (3)$$

which may be differentiated with respect to bc to give

$$dA/d(bc) = a_1 - [r(a_1 - a_2)10^{(a_1-a_2)bc}]/[1 + r \cdot 10^{(a_1-a_2)bc}] \qquad (4)$$

At low absorbances

$$dA/d(bc)_{bc\to 0} = \frac{a_1 + ra_2}{1+r} \qquad (5)$$

whereas when bc becomes large, $10^{a_1bc} \gg 10^{a_2bc}$ and the limiting slope is

$$dA/d(bc)_{bc\to\infty} = a_2. \qquad (6)$$

Thus, the limiting absorptivity at low absorbances is is a weighted average of a_1 and a_2 while, at higher absorbances, it is equal to a_2, the smaller of the individual absorptivities.

The theory for the correction of spectral bandpass errors has been developed by Hardy and Young [4], Eberhardt [5], and Broderson [6]. Rigorous calculation requires integration of the relation

$$A_{\text{obs}} = \log \frac{\int I_\lambda S_\lambda d\lambda}{\int I_\lambda S_\lambda 10^{-A} d\lambda} \qquad (7)$$

where I_λ is the incident intensity and S_λ is the spectral sensitivity of the detector. Equation (7) emphasizes the fact that the observed absorbance depends not only on the shape of the absorption curve, but also on the wavelength distribution of the source and the detector response. If $I_\lambda S_\lambda$ is constant over the wavelength interval used, approximate corrections for spectral bandpass may be calculated by assuming a Gaussian shape for the absorption peak and a triangular slit function for the instrument. Some calculations are given in table 1 based on these assumptions. The tabulated values of A_{obs}/A agree closely with those observed experimentally for A up to 1. As shown by Broderson [6] however, for values of RBW larger than 0.5, A_{obs}/A also depends on A and the above treatment is no longer applicable.

C. Stray Light

Stray light is defined as any light outside the spectral region isolated by the monochromator that reaches the detector. It is produced by scatter from the optics and

TABLE 1. Dependence of A_{obs} on spectral bandpass at an absorption maximum under idealized conditions (see text)

RBW[a]	A_{obs}/A	RBW[a]	A_{obs}/A
0.0100	0.99995	0.0800	0.9970
.0200	.9998	.0900	.9962
.0300	.9995	.1000	.9954
.0400	.9992	.2000	.9819
.0500	.9988	.3000	.9604
.0600	.9983	.4000	.9321
.0700	.9977	.5000	.8987

[a] Relative bandwidth: Ratio of spectral bandwidth to half bandwidth of fully resolved peak.

walls of the monochromator and is present in varying amounts in all spectrophotometers.

Stray light can lead to varied problems in spectrophotometry. Spurious absorption bands may arise in some cases. More frequently, however, deviations from the Lambert-Beer law are produced. These deviations are positive if the stray light is absorbed and negative if it is not.

Extensive literature exists on the detection, measurement and mimization of stray light. Several of the more useful papers are those by Hogness, Zscheile and Sidwell [7], Perry [8], Slavin [9], and Poulson [10] in which additional references may be found.

The amount of stray light present is proportionally large in those wavelength regions where the transmission of the monochromator, the source intensity or the detector sensitivity are relatively low. These regions, which should first be checked, are 200–230 nm and 600–700 nm. If a tungsten lamp must be used in the range 350–400 nm, a visible cutoff filter should always be employed.

The quantitative assessment of stray light requires the use of extremely sharp cutoff filters. Slavin [9] and Poulson [10] have described a number of liquid and solid filters that may be used. Aqueous solutions of the alkali halides, for example, are extremely useful in the ultraviolet. Care must be taken, however, that light leaks in the cuvet compartment are not contributing to the observed results. The apparent stray light will not vary appreciably with slitwidth if only instrumental stray light is present. In a quick test for light leaks, the apparent stray light will decrease rapidly as the slits are opened.

The stray light error encountered most frequently in spectrophotometry produces a decrease in an absorption peak and leads to negative deviations from the Lambert-Beer law. If it is assumed that none of the stray light is absorbed by the sample, the measured absorbance is related to the true transmittance by the expression [9]

$$A_{obs} = \log\,[(1-T)\,S+T]^{-1} \tag{8}$$

where S is the instrumental stray light expressed as a fraction. The effects of several levels of unabsorbed stray light on absorbance are given in table 2. Most samples absorb sufficiently in other regions of the spectrum to filter out a proportion of the stray light. Thus, indirect estimates of instrumental stray light based on deviations from linearity are generally low.

TABLE 2. Effect of unabsorbed stray light on absorbance

A	A_{obs}		
	$S=0.0001$	$S=0.001$	$S=0.01$
0.1	0.1000	0.0999	0.0989
.5	.4999	.4990	.4907
1.0	.9996	.9961	.9626
1.5	1.499	1.487	1.384
2.0	1.996	1.959	1.701

D. Nonparallel Incident Radiation

A perfectly parallel beam of radiation must come from a point source and can carry only an infinitesimal amount of energy. The light beam in a spectrophotometer always has some finite angular size. As a result, the average light path is greater than the perpendicular distance between the cuvet faces. Meehan [3] has considered the case in which the incident radiation is convergent or divergent in one dimension such as corresponds, respectively, to radiation focused by a cylindrical lens or leaving a narrow slit. If Θ and R are the angles of incidence and refraction, respectively, the path length of the extreme ray (fig. 2) is $b/\cos R$ and the absorbance of this ray is $A/\cos R$. Other rays enter at smaller angles and the observed absorbance is given by

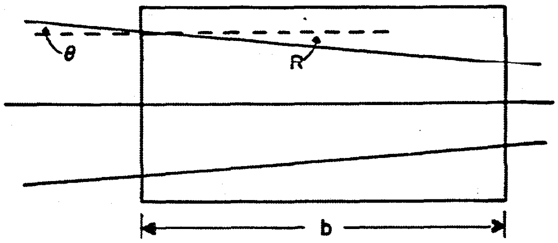


FIGURE 2. Nonparallel incident radiation [3]. Reprinted by permission of John Wiley & Sons, Incorporated.

$$A_{obs} = A/R_{max}\int_0^{R_{max}} 1/\cos R\,dR. \tag{9}$$

For this relation to hold, the incident beam must be uniform over its cross section. Evaluation of this integral for R_{max} of 2–10° ($\Theta_{max}=2.7°$–$13.4°$) gives the following percent errors in A (table 3). Thus, for angles of incidence up to 5 degrees, the absorbance error is less than 0.1 percent.

E. Multiple Reflections

On passage of light through a cuvet containing solvent or sample solution, some radiation is reflected

TABLE 3. Dependence of absorbance error on nonparallelism of incident radiation

R_{max}, degrees	Θ_{max}, degrees	Percent error in A
2	2.7	0.020
4	5.3	.081
6	8.0	.18
8	10.7	.33
10	13.4	.51

at each of the two air-glass and the two glass-liquid surfaces. For perpendicular incidence, the fraction f reflected on passing from a medium of refractive index n_1 to a second having refractive index n_2 is given by the Fresnel expression

$$f=\left[\frac{n_1-n_2}{n_1+n_2}\right]^2 \tag{10}$$

For an air-glass surface, $f=0.04$ and for a glass-liquid surface, $f=0.0035$. When more than one surface is involved, the effects of multiple reflections must be considered. The essential question when dealing with liquid filters is whether the solvent completely compensates for such reflections. According to Goldring et al. [11], it does not. They consider the case where the reflections from all surfaces perpendicular to the light beam on the two sides of the sample are grouped together to form two effective reflection coefficients, r_1 and r_2, as shown in figure 3. Considering only first and second order reflections, the observed absorbance is

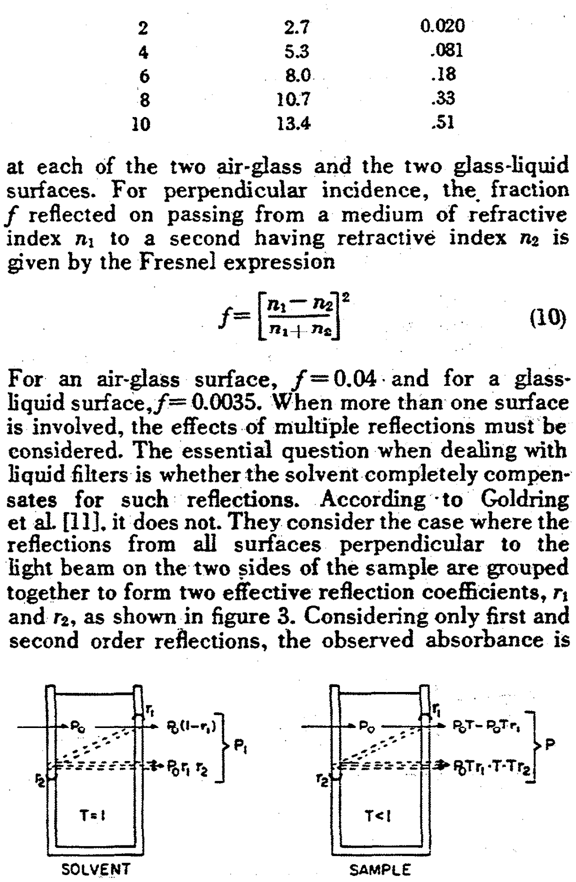


FIGURE 3. Internal multiple reflections [11]. Reprinted by permission of the American Chemical Society.

$$A_{obs}=\log\frac{P_1}{P_2}=\log\frac{P_0}{P_0T}\,\frac{(1-r_1+r_1r_2)}{(1-r_1+r_1r_2T^2)} \tag{11}$$

which, after transformation and series expansion, can be reduced to the following expression:

$$A_{obs}=A+0.4343\,(1-T^2)\,\frac{r_1r_2}{1-r_1}. \tag{12}$$

Considering only the reflections from the two cuvet faces and that $r_1=r_2=0.05$, the variation of A_{obs} with A is

A	0.1	0.2	0.5	1.0	2.0
A_{obs}	0.1004	0.2007	0.5010	1.0011	2.0011

Thus, because of internal multiple reflections, the measured absorbance should always be larger than the true absorbance with the percentage difference being greatest at low absorbances. Verification of these deviations is difficult experimentally. Goldring et al. [11], however, have suggested ways of minimizing reflection effects. These include positioning the cuvet at a small angle to the beam and stopping down the detector surface, immersing the cuvet in a fluid contained in a larger rectangular cuvet turned at an angle to the beam, or by using a cuvet with prismatic windows. In each case, the effective path length must be determined separately. Other alternatives consist of using cuvets constructed of absorbing materials or immersion of a glass filter into the sample. For a limited wavelength range, reflection errors can also be reduced by coating the external window surfaces with anti-reflection layers.

In addition to reflections from the cuvets, reflections from other surfaces must also be considered. Gibson [1] has pointed out that reflections may occur from the surfaces of the slit and detector and has suggested a means for checking this source of error. A thin glass plate is placed in its normal position at right angles to the beam and then at a small angle from this position. In the second position the reflected energy is directed out of the beam while scarcely affecting the true absorbance.

F. Cuvets

The majority of absorbance measurements are performed on solutions and, in such instances, the cuvet becomes an integral part of the measurement system. Presently, the uncertainty in the length of the light path can be a limiting factor in the determination of molar absorptivity. Nonparallelism of the end windows can be of even greater consequence. However, the effect can be minimized by using the same cuvet orientation for all measurements. The uncertainty in path length is nevertheless greater.

Cuvets are available in a variety of shapes and sizes and may be made of glass or silica. Construction remains largely an art and cuvets may vary considerably in their quality. Two methods of assembly are commonly used. These are: (1) fusion using only heat and (2) the use of intermediate, low-melting glasses. At present, each has its disadvantage. The first technique is the more desirable, but our experience, primarily with rectangular cuvets, indicates that the edges are not always completely fused. These surfaces exhibit capillarity and may become serious sources of contamination. Under such conditions, the cleaning of these cuvets is difficult, if not impossible. The use of low-melting fluxes on the other hand can produce strains because of differences in coefficients of expansion and cuvets so constructed are generally more fragile.

Cuvets are frequently offered in matched sets. This may be considered more of a convenience than a necessity since this terminology is normally used only to describe the transmission of the window material.

Path length and parallelism of end plates are of more fundamental importance. Unfortunately, no generally accepted tolerances have been established in this country for the construction of cuvets. The British Standards Institution, however, has published a set of specifications [12] which merit our consideration and possible adoption. They recommend that the path length be specified to ± 0.02 mm and the end windows be flat over a defined beam area to four Newton fringes per centimeter in mercury green light. It should be feasible for greater accuracy, especially for the 10 mm cuvets, to calibrate a limited number by appropriate metrology techniques to ± 0.01 mm.

III. Evaluation of Selected Liquid Filters

This section is a report of our efforts to develop well characterized liquid absorbance standards. Three general types of liquid filters are discussed. These are (1) individual solutions of high purity materials, (2) empirical mixtures and (3) aqueous solutions of organic dyes. Much of the emphasis has been placed on determining the optimum conditions for preparation of these filters and, once established, what the absorbing species or ionic compositions of these systems are. By combining this information with the instrumental considerations developed in the first part of this paper, it is hoped that the resulting data represent a step toward obtaining more meaningful absorptivity measurements.

A. Instrumentation

Absorbance measurements at a fixed wavelength were performed manually on a high precision double-beam spectrophotometer provided with a double monochromator. The accuracy of the photometric scale of this instrument was established with the NBS high-accuracy spectrophotometer described by Mavrodineanu [13]. The wavelength scale was checked with a mercury lamp. Potassium halide solutions [9, 10] were used to assess stray light in the ultraviolet.

Room temperature was maintained between 24 to 26 °C. The cuvet compartment and jacketed holders were thermostated by a circulating water bath. Copper-constantan thermocouples were used to measure temperature differences between the bath and the sample solution.

An electronic feeler gauge [13] was used to measure the path length of the 10 mm rectangular cuvets normally used. Solutions, assumed to obey Beer's law, were used for calibrating the 1 mm cuvets.

The accuracy of the microbalance was established with NBS-calibrated weights. Class A, 1-liter volumetric flasks were checked gravimetically. All dilutions were subsequently made by volume. To minimize errors from cell positioning, borosilicate, Pasteur-type pipets were used to transfer solutions to and from the cuvets.

B. Individual Solutions of High Purity Compounds

1. Potassium Dichromate. Numerous attempts have been made to use chormium (VI) solutions as ultraviolet absorbance standards. In the early studies [7, 14, 15, 16, 17] alkaline solutions of potassium chromate were preferred. More recently, potassium dichromate in slightly acidic media has been described [18, 19, 20]. The absorption spectra of these two systems are quite different with the latter giving the more desirable arrangement of maxima and minima (fig. 4).

The approximate composition of chromium (VI) solutions may be derived from the following equilibria:

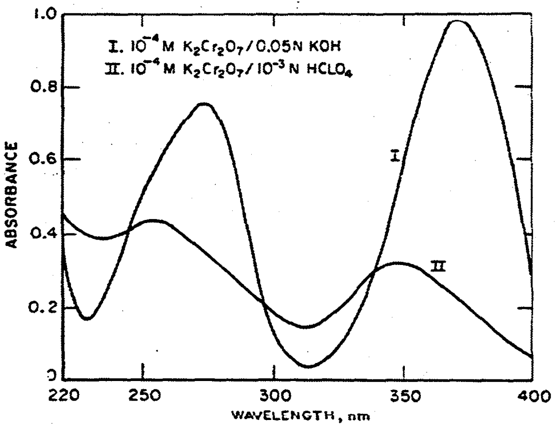


FIGURE 4. Absorption spectra of 10^{-4} M Cr (VI) in 0.05 N KOH and 10^{-3}N $HClO_4$.

$$H_2CrO_4 \overset{K_1}{=} H^+ + HCrO_4^- \quad (13)$$

$$HCrO_4^- \overset{K_2}{=} H^+ + CrO_4^= \quad (14)$$

$$2HCrO_4^- \overset{K_3}{=} Cr_2O_7^= + H_2O. \quad (15)$$

Some values reported for the equilibrium constants K_1, K_2, and K_3 at 25 °C are as follows:

$K_1 = 0.16$ [21]

$K_2 = 3.2 \times 10^{-7}$ [22] and 3.0×10^{-7} [23]

$K_3 = 43.7$ [21], 35.5 [24] and 33.0 [25].

Thus, at a pH greater than 10, chromium (VI) exists almost entirely as $CrO_4^=$ ions while, in weakly acidic solutions, the predominant species is $HCrO_4^-$ which partially dimerizes to $Cr_2O_7^=$. The amount of dimer will increase with increasing chromium concentration. The formation of H_2CrO_4 or $CrO_4^=$ can be essentially eliminated by maintaining the acidity near pH 3.

Much of the uncertainty that has arisen from the use of acidic potassium dichromate solutions as potential absorbance standards can be attributed to the dimerization reaction (eq (15)). It is primarily this equilibrium which leads to the observed deviations from Beer's law. Because of the range of values reported for K_3, one of the first objectives of the present study was to redetermine this constant. The spectrophotometric method was chosen and is described in some detail below.

a. Spectrophotometric Determination of the Dimerization Constant for: $2HCrO_4^- = Cr_2O_7^= + H_2O$. If $HCrO_4^-$ and $Cr_2O_7^=$ are the only Cr(VI) species present with molar absorptivities ϵ_1 and ϵ_2, respectively, the apparent molar absorptivity, ϵ_m, will be given by

$$\epsilon_m = (1-\alpha)\epsilon_1 + 1/2 \cdot \alpha\epsilon_2 \tag{16}$$

where α is the fraction of total chromium in the dichromate form. From eq (15) the thermodynamic equilibrium constant of the dimerization reaction is

$$K_3 = \frac{[Cr_2O_7^=]}{[HCrO_4^-]^2} \cdot \frac{\gamma_2}{\gamma_1^2} \tag{17}$$

or, in terms of α

$$K_3 = \frac{\alpha}{2(1-\alpha)^2 Cr_T} \cdot \frac{\gamma_2}{\gamma_1^2} \tag{18}$$

where γ_1 and γ_2 are the activity coefficients of $HCrO_4^-$ and $Cr_2O_7^=$, respectively, and Cr_T is the total chromium concentration. Because the ionic strength never exceeded 0.01, the activity coefficients may be treated by the Debye-Hückel expression [25]

$$-\log \gamma_i = \frac{AZ_i^2 I^{1/2}}{1+I^{1/2}} \tag{19}$$

where Z is the ionic charge, I is the ionic strength and A has a value of 0.509 l/mol at 25 °C. Equations (16) and (18) can be rewritten to give, respectively,

$$\epsilon_m = \alpha(1/2\,\epsilon_2 - \epsilon_1) + \epsilon_1 \tag{20}$$

and

$$\log K_3 = \log \frac{\alpha}{2(1-\alpha)^2 Cr_T} - \frac{2AI^{1/2}}{1+I^{1/2}} \cdot \tag{21}$$

Assuming a value of K_3, one can calculate from eq (21) the α values at various total chromium concentrations. If the choice of K_3 is correct, the measured values of ϵ_m should lie on a straight line of slope $(1/2\,\epsilon_2 - \epsilon_1)$ and intercept ϵ_1, as seen from eq (20). The best value of K_3 is then determined by the method of least squares.

1. *Experimental Procedure*

The chromium (VI) solutions used in the subsequent studies were prepared from NBS SRM–136c, potassium dichromate, which had been dried at 110 °C for two hours. The distilled water was shown to be free of reducing impurities by titration with a dilute potassium permanganate solution. Perchloric acid was used to maintain the pH at 3.0 ± 0.1.

The optimum wavelengths for the determination of K_3 were chosen as follows: Two solutions were prepared, one containing 391 mg $K_2Cr_2O_7$/l and the other, 40.0 mg $K_2Cr_2O_7$/l. The former was transferred to a 1.023 mm cuvet and the latter to a 10.00 mm cuvet. A differential scan was then made on a recording spectrophotometer equipped with a 0–0.1 absorbance (full scale) slidewire, using the less concentrated solution as the reference. The resulting spectrum is shown in figure 5. Relatively large differences in absorbance are seen in the wavelength regions near 275 and 385 nm even though the total number of chromium atoms in each light path is the same. Thus, it is in these two regions that the largest deviations from Beer's law should be observed. Three wavelengths were subsequently selected in each region for the determination of K_3–390, 385 and 380 and 280, 275 and 270 nm, respectively.

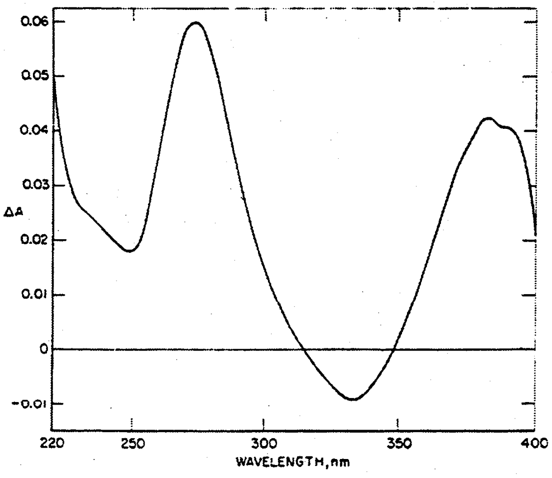


FIGURE 5. Difference spectrum of 391 mg $K_2Cr_2O_7$/l in 1.023 mm cuvet versus 40.0 mg $K_2Cr_2O_7$/l in 10.00 mm cuvet; pH = 3.

2. *Results*

The apparent molar absorptivities calculated from absorbance measurements on the four chromium concentrations used for the determination of K_3 are given in table 4. Using the method of least squares, these ϵ_m values were plotted against the α fractions calculated from eq (21) for various assumed values of K_3. The molar absorptivities were weighted according to the reproducibility of the absorbance measurements. A computer was used for all calculations.

A typical graph of the residual standard deviation of the experimental ϵ_m values from the best straight line fit is shown in figure 6 for K_3 values of 28 through 36. The minimum in this curve is the best estimate of K_3 and, for the case shown, is 32.6. The K_3 values at the six wavelengths are summarized in table 5.

TABLE 4. Apparent molar absorptivities, ϵ_m, of $K_2Cr_2O_7$ solutions at 25 °C; pH = 2.9 ($HClO_4$)

$K_2Cr_2O_7$ Conc. mg/l	ϵ_m l/mol^{-1} cm^{-1}					
	390 nm	385 nm	380 nm	280 nm	275 nm	270 nm
100.06	589.2	748.7	865.1	1579.3	1718.6	1881.0
400.48	714.1	878.6	997.8	1758.2	1914.4	2069.8
699.86	806.2	973.8	1094.0	1890.0	2053.5	2208.0
1000.27	875.9	1045.6	1166.5	1988.2	2161.5	2313.6

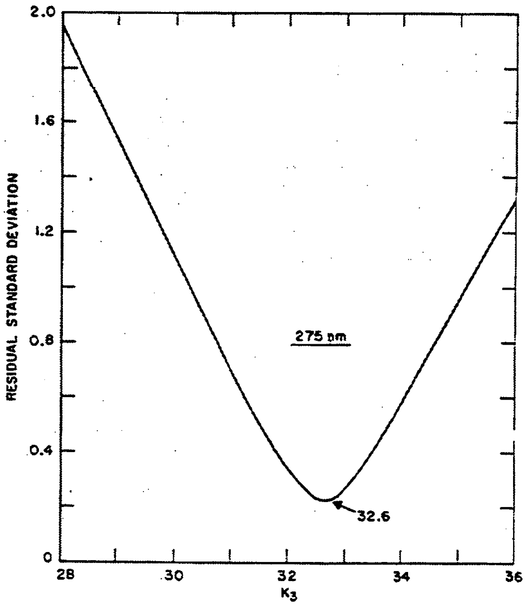


FIGURE 6. Least squares determination of K_3.

TABLE 5. K_3 values at 25 °C as determined at several wavelengths

λ, nm	K_3
390	32.4
385	33.3
380	33.8
280	33.5
275	32.6
270	31.6
Avg.	32.9
Std. Dev.	0.8
Rel. Std. Dev.	2.5%

b. Absorption Spectra of $HCrO_4^-$ and $Cr_2O_7^=$. The absorbances of three solutions containing nominally 20, 40, and 60 mg $K_2Cr_2O_7$/l (pH = 3.0 ± 0.1) were measured in a 10.00 mm cuvet at 5 nm increments over the wavelength range of 220–400 nm. A close approximation of $\epsilon(HCrO_4^-)$ was obtained by extrapolating the apparent molar absorptivities to zero chromium concentration. This extrapolation is simplified since the variation of α is nearly linear over this concentration range. Similar absorbance measurements were performed on three solutions containing 1000 mg $K_2Cr_2O_7$/l using a 1.023 mm cuvet and the corresponding α value was calculated from eq (21), with $K_3 = 32.9$. The first approximations of $1/2 \cdot \epsilon(Cr_2O_7^=)$ values were obtained by substituting the extrapolated $\epsilon(HCrO_4^-)$ results and the above α value into eq (20). The $\epsilon(HCrO_4^-)$ and $1/2 \cdot \epsilon(Cr_2O_7^=)$ values were then refined by successive approximations. The calculated absorption spectra of these two ions are shown in figure 7.

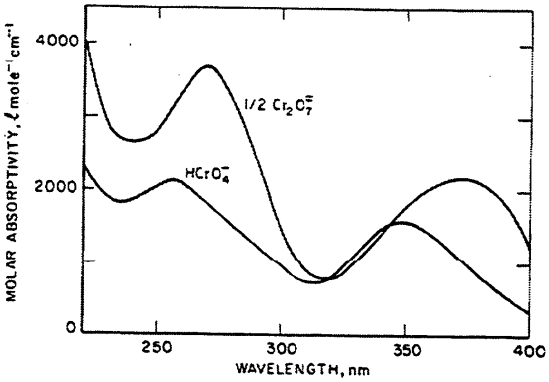


FIGURE 7. Absorption spectra of $HCrO_4^-$ and $Cr_2O_7^=$.

c. Molar Absorptivities of $HCrO_4^-$ and $Cr_2O_7^=$ at 350, 313, 257, and 235 nm. As shown previously in figure 4, the absorption spectrum of weakly acidic solutions of $K_2Cr_2O_7$ exhibit maxima at 350 and 257 nm and minima at 313 and 235 nm. These maxima and minima do not shift significantly over the concentration range of 20–100 mg $K_2Cr_2O_7$/l. This is considered a practical range for most applications since it yields absorbances of about 0.1–1.5 for a 10 mm cuvet.

The molar absorptivities of $HCrO_4^-$ and $Cr_2O_7^=$ at 350, 313, 257, and 235 nm were determined by the procedure described above and are given in table 6. The $\epsilon(HCrO_4^-)$ values are estimated to be accurate to ± 0.5 percent. Because of the imprecision of the K_3 determination, the uncertainty of the dichromate molar absorptivity values may be as large as ±5 percent.

TABLE 6. Molar absorptivities, ϵ, of $HCrO_4^-$ and $Cr_2O_7^=$ at 25 °C

	ϵ, l mol^{-1} cm^{-1}			
	350 nm	313 nm	257 nm	235 nm
$HCrO_4^-$	1576	711	2100	1804
$1/2\,Cr_2O_7^=$	1788	823	3156	2688

Table 7 shows the agreement between the experimental and calculated apparent molar absorptivities for five concentrations of potassium dichromate in dilute perchloric acid (pH = 2.92 ± 0.02). The experimental values are the averages obtained on five individual samples measured at each concentration. The 95 percent confidence limits for any given set of measurements was 0.1 percent. The α values used are also given. These are seen to vary nearly linearly over the range of chromium concentrations employed.

d. Optimum pH and Choice of Acid. The chromium (VI) solutions used in this study were prepared in dilute perchloric acid media having a pH of about 3. This acidity was selected because the existing equilibria data indicated this pH limited the formation of either H_2CrO_4 or $CrO_4^=$ to less than 0.1 percent of the total Cr (VI) concentration. Perchloric acid was chosen instead of sulfuric to prevent the formation of chromate-sulfate complexes. Tong and King [24] and Davies and Prue [25] noted slight differences in the absorbances of dilute perchloric and sulfuric acid solutions after correcting for differences in ionic strength. They attributed these differences to mixed complex formation. More recently, Haight et al. [26] have shown that the conversion of $HCrO_4^-$ to $CrSO_7^=$ is quantitative in 1 *M* HSO_4^- solutions and that absorption spectrum of this complex is significantly different from the $HCrO_4^-$ spectrum.

Because most of the previous work reported in literature has been done in sulfuric acid, it was considered of interest to intercompare these two systems. The results are shown in each acid between pH 2 and 3. At 350 nm the results obtained in perchloric acid are consistently 0.1–0.2 percent higher than in sulfuric; at 235 nm, they are lower. The relatively large increase in absorptivity at 235 nm at pH 1.90, together with the above considerations, suggest the formation of additional chromium (VI) species in systems employing dilute sulfuric acid solutions. (See table 8.)

e. Effect of Temperature. The absorbances of a solution containing 60 mg $K_2Cr_2O_7$/l in dilute perchloric acid (pH = 3) were measured over the temperature range of 17 to 37 °C. The percentage change in absorbance for wavelengths of 350, 313, 257, and 235 nm is shown in figure 8. The results are uncorrected for

TABLE 7. Experimental and calculated values (E, C) of the apparent molar absorptivity of $K_2Cr_2O_7$ at 25 °C; pH = 2.9($HClO_4$)

$K_2Cr_2O_7$ Conc. mg/l	α		ϵ_m, l mol^{-1} cm^{-1}			
			350 nm	313 nm	257 nm	235 nm
20.22	0.0090	E	1577.6	713.5	2113.6	1814.7
		C	1577.9	711.9	2108.7	1812.1
		Diff.	−0.3	+1.6	+4.9	+2.6
40.09	0.0178	E	1579.9	713.3	2120.8	1820.2
		C	1579.8	713.0	2118.8	1819.7
		Diff.	+0.1	+0.3	+2.0	+0.5
60.12	0.0262	E	1581.4	713.8	2127.2	1827.1
		C	1581.5	713.8	2126.7	1827.2
		Diff.	−0.1	0.0	+0.5	−0.1
80.17	0.0344	E	1584.0	715.0	2137.6	1835.8
		C	1583.3	714.8	2135.3	1834.4
		Diff.	+0.7	+0.2	+2.3	+1.4
99.92	0.0423	E	1585.5	715.6	2144.0	1841.5
		C	1584.0	715.0	2143.7	1841.4
		Diff.	+1.5	+0.6	+0.3	+0.1

TABLE 8. Apparent absorptivities, *a*, of sulfuric and perchloric acid solutions of $K_2Cr_2O_7$ at 25°C; $K_2Cr_2O_7$ conc. = 0.050 g/l

		a, l g^{-1} cm^{-1}			
	pH	350 nm	313 nm	257 nm	235 nm
H_2SO_4	1.90	10.71_0	4.83_7	14.44_4	12.43_7
	1.98	10.71_2	4.83_9	14.44_0	12.41_8
	2.20	10.72_2	4.83_9	14.42_9	12.40_2
	3.00	10.73_3	4.84_4	14.42_7	12.38_1
$HClO_4$	1.99	10.72_7	4.84_8	14.44_8	12.39_6
	3.08	10.74_0	4.84_5	14.43_4	12.38_3

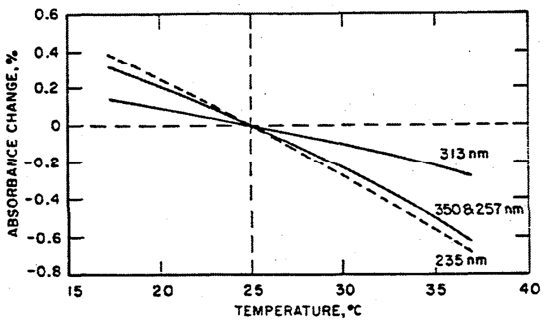


FIGURE 8. Change in absorbance with temperature for acidic solutions of $K_2Cr_2O_7$: pH = 3 ($HClO_4$).

thermal expansion of the solvent. The absorption minimum at 313 nm exhibits the smallest temperature dependence (−0.02 percent per °C) while the maxima at 350 and 257 nm and the minimum at 235 nm have temperature coefficients of −0.05 percent per °C over the range 20 to 30 °C.

f. $K_2Cr_2O_7$ in 0.5 M Na_2HPO_4 and 0.05 M KOH. To overcome some of the difficulties in handling strongly alkaline chromate solutions, Johnson [27] has recommended dissolving $K_2Cr_2O_7$ in 0.05 M Na_2HPO_4 at pH 9. The apparent molar absorptivities obtained in this medium and in 0.05 M KOH are given in table 9. As seen from the data, both systems appear to exhibit deviations from Beer's law of about three parts per thousand over the concentration range studied. Although this deviation is the same magnitude and direction predicted from uncompensated internal multiple reflections discussed previously, we have not been able to demonstrate experimentally that such reflections are responsible for the observed results. Studies in which the cuvet windows are coated with various antireflection layers are continuing however.

TABLE 9. Apparent molar absorptivity of potassium chromate in 0.05 *M* Na_2HPO_4 and 0.05 *M* KOH at 25 °C

0.05 *M* Na_2HPO_4

K_2CrO_4 Conc., $M \times 10^5$	pH	ϵ_m, l mol^{-1} cm^{-1} 373 nm	ϵ_m, l mol^{-1} cm^{-1} 274 nm
7	9.2	482_7	370_3
14	9.1	482_0	369_7
21	9.1	481_3	369_2
0.05 *M* KOH			
7	....	483_0	370_5
14	....	482_4	369_8
21	....	481_4	369_1

From a consideration of the equilibrium data, the absorbances in the two systems should be nearly identical. The ionic strength of the 0.05 *M* Na_2HPO_4 is 0.15 and, from Neuss and Rieman's work [22], the ionization constant for the reaction $HCrO_4^- = H^+ + CrO_4^=$ is approximately 9×10^{-7} mol/liter. Thus, at pH 9.1, only about 1 in 1000 chromium atoms is present as $HCrO_4^-$. Since this species is less absorbing than $CrO_4^=$ at 373 and 274 nm, the apparent molar absorptivities in 0.05 *M* Na_2HPO_4 should agree with those obtained in 0.05 *M* KOH to within 0.1 percent.

The absorbance of alkaline chromate solutions decreases with increasing temperature at both absorption peaks. Between 17 and 37 °C, the temperature coefficient at 373 nm is −0.09 percent per °C; at 274 nm, it is −0.06 per °C.

2. Potassium Nitrate. Aqueous solutions of potassium nitrate exhibit a nearly symmetrical absorption peak with λ_{max} at 302 nm. This system has been studied extensively by Vandenbelt [14] and by the British Photoelectric Spectrometry Group in two collaborative tests [28, 29]. Molar absorptivities reported vary from 7.06 to 7.20 liter mol^{-1} cm^{-1}. This range of values undoubtedly results in part from the fact that KNO_3 solutions deviate significantly from Beer's law.

Careful reexamination of this system has yielded the following apparent molar absorptivities (table 10). These are seen to decrease rather markedly with increasing potassium nitrate concentration. Several factors conceivably contribute to this behavior. In addition to possible multiple reflection effects already discussed some ionic interactions are undoubtedly possible in this system because of the relatively high concentrations of solute required coupled with the fact that the N−O bond in the nitrate ion has some polar character.

The potassium nitrate used in the above study was analyzed for commonly encountered impurities. After drying at 110 °C, the water content was less than 0.02 percent. Flame emission analyses [31] indicated the following levels of alkali and alkaline earth impurities (in parts-per-million): Li (< 0.2); Rb (13); Mg (0.2); Ca (1); Sr(4) and Ba (< 2).

At 302 nm, the absorbance of KNO_3 solutions decreases with increasing temperature. Over the range 17 to 37 °C, the temperature coefficient is −0.14 percent per °C.

Solutions stored for up to six months showed no measurable change (≤ 0.1 percent) in absorbance. Edisbury [29] has cautioned, however, that some solutions may develop bacterial whiskers on standing. Sterilization may be effected by prior boiling of the distilled water.

TABLE 10. Apparent molar absorptivity, ϵ_m, of aqueous solutions of potassium nitrate at 25 °C

	ϵ_m, l mol^{-1} cm^{-1}
KNO_3 Conc., *M*	302 nm[a]
0.028	7.16_0
.056	7.14_2
.084	7.12_7
.112	7.10_6
.140	7.09_1

[a] Spectral bandpass = 1.0 nm

3. Potassium Hydrogen Phthalate. High purity potassium hydrogen phthalate (KHP) is readily available. It is presently issued by the National Bureau of Standards as a primary acidimetric standard. Its use in the preparation of buffer solutions of known pH is well established. It has also been used as a spectral standard in the comparative evaluation of spectrophotometers [11, 32].

The absorbance of KHP solutions is dependent on acidity, as shown in figure 9. In the present study, dilute perchloric acid solutions were employed to minimize changes in ionic composition as a function of pH. Acid concentrations much above 0.1 *N* could not be used because of the precipitation of potassium perchlorate. Subsequently, all solutions were prepared to contain 10 ml of perchloric acid per liter with a pH of 1.3.

Ringbom [21] has given the first ionization constant of phthalic acid as 1.6×10^{-3} at 25 °C at an ionic strength of 0.1. At the pH used, the absorbing system consisted of 97 percent phthalic acid and 3 percent hydrogen phthalate ions. Table 11 summarizes the absorptivity values obtained on these solutions.

Absorbance decreases at the 275.5 nm maximum and increases at the 262 nm minimum with increasing temperature. Over the range 17 to 37 °C the temperature coefficients are −0.05 percent and +0.05 percent per °C, respectively.

Perchloric acid solutions of KHP (NBS–SRM 84g) were examined for possible fluorescence, using a high sensitivity spectrofluorometer. With 280 nm excitation, a faint fluorescence was detected with λ_{max} at approximately 350 nm. The possible effects of this fluorescence on the absorbance data remain to be established.

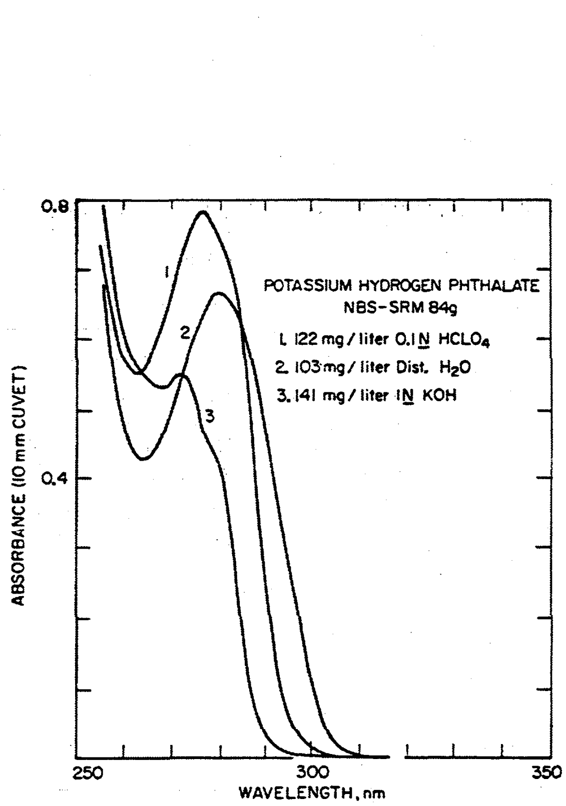


FIGURE 9. Absorption spectra of KHP solutions at different acidities.

TABLE 11. Apparent absorptivity, a, of potassium hydrogen phthalate in dilute perchloric acid at 25 °C; 10 ml $HClO_4$/liter

NBS SRM 84g Conc., g/l	a, l g^{-1} cm^{-1}	
	275.5 nm	262 nm
0.034	6.33_2	4.49_4
.142	6.31_9	4.48_9

4. Cobalt and Nickel Sulfates and Perchlorates. Aquo cobalt (II) and nickel (II) ions have relative broad absorption peaks with λ_{max} at 512 and 394 nm, respectively (fig. 10). Solutions of either may serve as useful absorbance standards. Most of the attention to date, however, has been directed at cobalt sulfate which was first recommended by Gibson [1].

In this study, the apparent molar absorptivities of $Co(H_2O)_6^{+2}$ and $Ni(H_2O)_6^{+2}$ were examined in sulfate and perchlorate media. All solutions were prepared from the high purity metals by dissolution in H_2SO_4–HNO_3 or $HClO_4$–HNO_3 acids. Nitrate was removed by repeated fuming until the acidity was reduced so that on dilution to volume, a pH of 1 could be obtained. The results obtained in the two acid media are given in table 12. The values in sulfate solution are significantly higher than in perchlorate media for both cobalt and nickel, suggesting that some complexation of these ions by $SO_4^=$ or HSO_4^- has occurred. A differ-

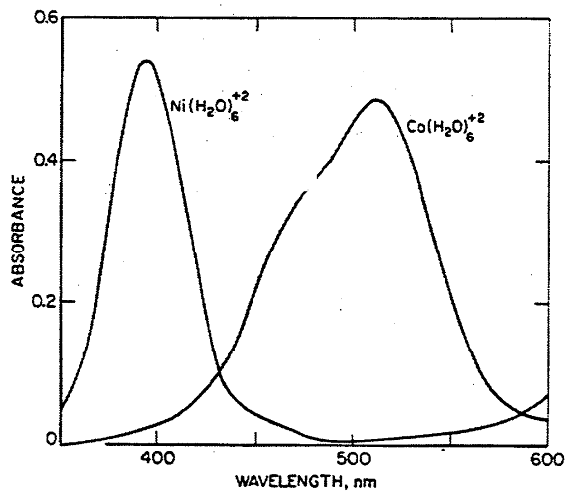


FIGURE 10. Absorption spectra of $Ni(H_2O)_6^{+2}$ and $Co(H_2O)_6^{+2}$.

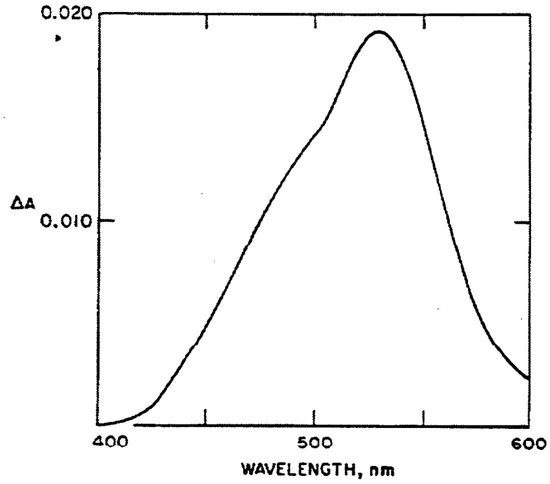


FIGURE 11. Differential spectrum of cobalt sulfate versus cobalt perchlorate; 10 mm cuvets; pH = 1.

TABLE 12. Apparent molar absorptivities, ϵ_m of acidic solutions of cobalt and nickel ions at 25 °C; pH = 1.0

Co Conc. g/l	ϵ_m (512 nm) l mol^{-1} cm^{-1} Sulfate	ϵ_m (512 nm) l mol^{-1} cm^{-1} Perchlorate
2.4	4.88_1	4.80_3
12.0	4.86_9	4.79_1

Ni Conc. g/l	ϵ_m (394 nm) l mol^{-1} cm^{-1} Sulfate	ϵ_m (394 nm) l mol^{-1} cm^{-1} Perchlorate
2.3	5.16_7	5.09_0
11.5	5.15_4	5.07_7

ential spectrum of 0.2000 molar cobalt solution in sulfate against the same cobalt concentration in perchlorate media is shown in figure 11. In sulfate solution, the formation of a second cobalt species with $\lambda_{max} = 528$ nm is indicated. Similar behavior was also observed for nickel.

The absorbances of cobalt and nickel solutions at their maxima are temperature dependent and increase with increasing temperature. Between 17 and 37 °C the temperature coefficients are 0.18 and 0.14 percent per °C at 512 and 394 nm, respectively. To explain their nuclear magnetic resonance data on cobalt(II) solutions, Swift and Connick [33] have suggested that the octahedral $Co(H_2O)_6^{+2}$ complex is in equilibrium with the tetrahedral $Co(H_2O)_4^{+2}$ complex and that formation of the latter is favored by increasing temperature. By analogy with halide complexes, the tetrahedral configuration would be expected to be approximately one hundred times more absorbing than the octahedral complex. A very small shift in this equilibrium could result in a relatively large change in absorbance and thereby account for the large temperature coefficient of this system. The same explanation may also be valid for nickel solutions.

C. Liquid Filters of Improved Optical Neutrality

Two approaches have been used in an attempt to improve the optical neutrality and extend the wavelength range of liquid filters. One is based on the use of empirical mixtures, while the other employs water-soluble, organic "black" dyes.

In 1946, Thomson [34] described the preparation of a grey inorganic solution consisting of chromic, cobaltous and cupric sulfates and potassium dichromate. This solution, however, does not transmit below 300 nm. To extend the wavelength range farther into the ultraviolet, a second exploratory solution (henceforth referred to as the NBS composite) was prepared in which the cupric and potassium dichromate components of the Thomson mixture were replaced by *p*-nitrophenol. The absorption spectra of these solutions are compared in figure 12 to a commonly used glass filter (Chance ON–10). The NBS composite was prepared in dilute sulfuric acid and had a pH of 1. No fluorescence was observed under these conditions.

Over the range 250–600 nm, the NBS composite exhibits several broad maxima and minima whose absorbances are less dependent on spectral bandpass than the Chance ON–10 glass. Examples are given in figure 13 at two wavelengths for each.

The possible use of either Thomson solution or the NBS composite as an absorbance standard is limited by two factors, both of which arise from the presence of chromium (III) in these mixtures. First,

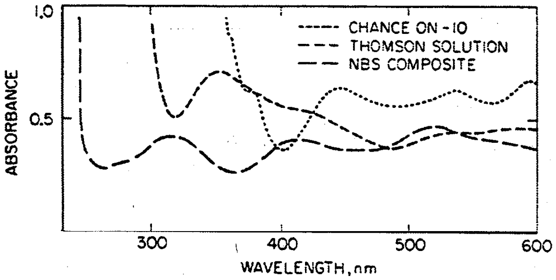


FIGURE 12. Absorption spectra of Thomas solution, an NBS composite and a Chance ON-10 glass.

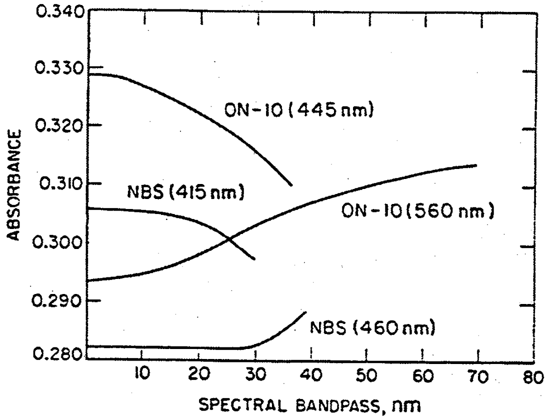
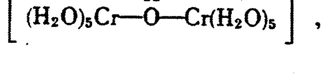


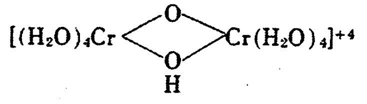
FIGURE 13. Variation of absorbance with spectral bandpass for NBS composite and Chance ON-10 glass.

an initial "aging" of 6–8 weeks period is needed in order to achieve adequate spectral stability at room temperature. Secondly, and even more critical for our purpose, is that the absorbances of the aged solutions exhibit a nonreversible temperature dependence. They must therefore always be stored at the temperature at which they were aged.

Hall and Eyring [35] have studied the constitution of chromic salts in aqueous solution and have suggested that during the aging process $Cr(H_2O)_6^{+3}$ ions polymerize to yield species of the type

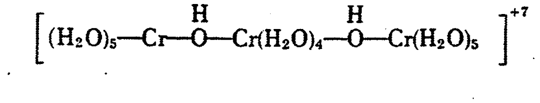


$$\left[(H_2O)_5Cr\overset{H}{-O-}Cr(H_2O)_5 \right]^{+5},$$



$$[(H_2O)_4Cr\langle\overset{H}{O}\rangle\langle\underset{H}{O}\rangle Cr(H_2O)_4]^{+4}$$

and



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

in which the observed increase in acidity is explained by the formation of —OH bridges. Recent studies [36] have shown that temperature, initial pH and the type of anion present significantly affect the equilibrium composition. Although refluxing can reduce the aging period from several months to several hours, this is of no practical advantage since the high temperature products, on cooling, are no longer stable.

In addition to the investigation of empirical mixtures, several water-soluble organic dyes have also been studied. Some typical absorption spectra are shown in figure 14. All of these dyes have high absorptivities and the concentrations used were of the order of 50 milligrams per liter.

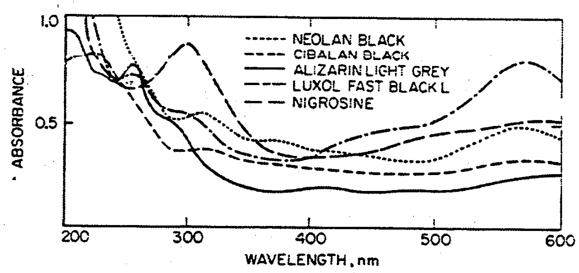


FIGURE 14. Absorption spectra of aqueous solutions of selected dyes.

Cibalan Black [37], Neolan Black [37] and Alizarin Light Grey [38] were selected for further evaluation. Aqueous solutions of these dyes were found insensitive to pH over the range 2–9, not affected by heat and relatively light fast. Solutions continuously exposed to fluorescent light exhibited an absorbance change of about 1 percent over a period of two months. These initial results appeared sufficiently promising to warrant purification and more extensive testing. Members of the Organic Chemistry Section have assisted in this effort. The purification of these dyes has proven extremely difficult, however, and yields of only a few hundred milligrams of each have been obtained. Much larger quantities are needed if these dyes are to be used as Standard Reference Materials. More efficient purification procedures are presently being sought.

D. Standard Reference Material (SRM) 931

The first liquid absorbance standard to be issued by the National Bureau of Standards is SRM 931. The preparation and certification of this Standard Reference Material are described below.

The filters were prepared by dissolving high purity cobalt and nickel in a mixture of nitric and perchloric acids. The weights of cobalt and nickel were chosen so that the absorbances of their aquo complexes were approximately equal at their absorption maxima. The absorbance of nitrate was adjusted to a comparable level by evaporation and subsequent addition

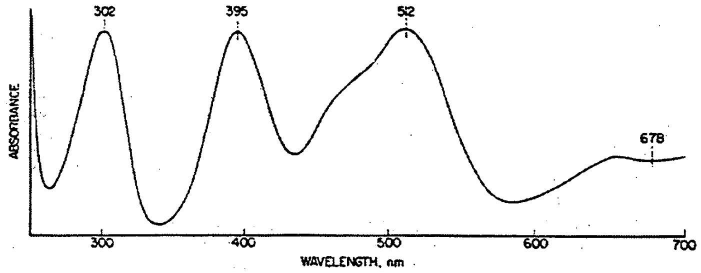


FIGURE 15. Typical absorption spectrum of SRM 931.

of small amounts of nitric acid. The final pH of these mixtures was about 1.

An absorption spectrum of a typical filter is shown in figure 15. The peaks at 302 and 512 nm are due to absorption by NO_3^- and $Co(H_2O)_6^{+2}$, respectively. The peak at 395 nm and the plateau at 650–700 nm are due to $Ni(H_2O)_6^{+2}$.

The filters are supplied in 10 ml ampoules which have been prescored for easy opening. An SRM unit consists of three sets of filters, each set containing a "Blank" (0.1N perchloric acid) and three absorbance levels of the filter. Each set is packaged in an individual tray for added convenience.

The filters were calibrated by randomly selecting ampoules from each lot. The certified absorbances are as follows:

TABLE 13. Certification of NBS SRM 931

Net absorbance[a]

Filter	Wavelength and (Bandpass), nm			
	302(1.0)	395(1.7)	512(2.0)	678(6.5)
"A"–'Blank'	0.307±0.003	0.304±0.003	0.303±0.003	0.115±0.002
"B"–'Blank'	0.608±0.005	0.605±0.005	0.606±0.005	0.229±0.003
"C"–'Blank'	0.906±0.007	0.907±0.007	0.911±0.007	0.345±0.003

[a] Net absorbances "A"–'Blank,' "B" –'Blank,' and "C"–'Blank' were determined using 10.00 mm cuvets at 25.0 °C.

The uncertainties are given at the 95 percent confidence level and include a possible systematic error of ±0.5 percent.

Absorbances are certified at 25.0 °C. Absorbances at other temperatures in the range 17 to 37 °C may be calculated using the formula:

$$A_t = A_{25}[1 + C_A(t-25)]$$

where: A_t=Absorbance at temperature t (°C)
A_{25}=Absorbance certified at 25.0 °C
C_A=Fractional change in absorbance per °C

The values of C_A at the four wavelengths are given below.

λ, nm	C_A
302	−0.0014
395	+0.0014
512	+0.0018
678	+0.0014

The absorbances of these filters will also depend on the spectral bandpass used. To insure that the measured absorbances do not differ by more than ±0.1 percent from the true values, the effective spectral bandpass should not exceed 1.5, 2.0, 3.3, and 8.5 nm at 302, 395, 512, and 678 nm, respectively.

IV. Conclusion

The availability of well characterized materials from which the analyst prepares his own solutions is preferable to the issuance of ampouled liquids. Although the former are not as convenient to use, they offer potentially greater accuracy because of their better stability.

The potassium dichromate and potassium hydrogen phthalate used in this study are issued by NBS as primary oxidimetric and acidimetric standards (SRM 136c and 84g, respectively). The potassium nitrate is presently issued as a thermal analysis standard (SRM 756). A product of comparable purity may be obtained by repeated recrystallization of reagent-grade material from distilled water. The cobalt and nickel solutions are best prepared from the high purity metals which are commercially available.

To certify absorptivities for these materials, one must resolve the question as to how well these systems obey Beer's law. For acidic potassium dichromate, the increased dimerization of $HCrO_4^-$ to $Cr_2O_7^{=}$ with increasing chromium concentration primarily accounts for the relatively large deviations observed. With potassium nitrate, one can only speculate that ionic interactions are enhanced at the higher concentrations. In neither instance are these deviations sufficiently large to limit the usefulness of these materials as liquid absorbance standards. Such deviations do require that the absorptivity data be used with proper precautions.

Additional studies are needed to explain the 0.2–0.3 percent deviations from Beer's law obtained for the various concentrations of alkaline chromate, potassium hydrogen phthalate, cobalt perchlorate and sulfate, and nickel perchlorate and sulfate. Although internal multiple reflection effects appear to be of the proper magnitude to account for these differences, initial attempts to measure such effects with magnesium fluoride-coated cuvets have not been successful. While extremely durable, magnesium fluoride reduces reflections by about 50 percent and more efficient coatings will probably be needed to resolve this problem. If internal reflections are responsible for the observed deviations, the absorptivity data given should not be corrected when using liquid absorbance standards for checking the accuracy of the photometric scale since these reflections are included in every measurement. However, in the determination of molar absorptivity, which is considered a fundamental property of the material, appropriate corrections should be applied. Until this question can be resolved, it is recommended that these materials be used at concentrations which yield an absorbance of at least 0.5 when measured in a 10 mm cuvet. With this restriction, it is believed that the uncertainty of the absorptivity data does not exceed ±0.5 percent at the 95 percent confidence level.

The authors wish to express their appreciation to Radu Mavrodineanu for providing the calibrated Schott filters and to David S. Bright for performing the computer calculations on the $HCrO_4^-/Cr_2O_7^{=}$ equilibrium.

V. References

[1] Gibson, K. S., Nat. Bur. Stand. Circular 484 (1949).

[2] Harrison, G. R., M.I.T. Wavelength Tables (John Wiley & Sons, Inc., New York, N.Y., 1969).

[3] Meehan, E. J., 'Treatise on Analytical Chemistry', Kolthoff, I. M., and Elving, P. J., Editors, Part 1, Volume **5**, Chapter 54 (John Wiley & Sons, Inc., New York, N.Y., 1964).

[4] Hardy, A. C., and Young, F. M., J. Opt. Soc. Am. **39**, 365 (1949).

[5] Eberhardt, W. H., J. Opt. Soc. Am. **40**, 172 (1950).

[6] Broderson, S., J. Opt. Soc. Am. **44**, 22 (1954).

[7] Hogness, T. R., Zscheile, F. P., and Sidwell, A. E., J. Phys. Chem. **41**, 379 (1937).

[8] Perry, J. W., Photoelectric Spectrometry Group Bull. **3**, 40 (1950).

[9] Slavin, W., Anal. Chem. **35**, 561 (1963).

[10] Poulson, R. E., Appl. Opt. **3**, 99 (1964).

[11] Goldring, L. S., Hawes, R. C., Hare, G. H., Beckman, A. O., and Stickney, M. E., Anal. Chem. **25**, 869 (1953).

[12] British Standard 3875, British Standards Institution (1965).

[13] Mavrodineanu, R., Nat. Bur. Stand. (U.S.), Tech. Note 584, 178 pages (1971), O. Menis and J. I. Schultz, Eds.

[14] Vandenbelt, J. M., Forsyth, J., and Garrett, A., Anal. Chem. **17**, 235 (1945).

[15] Haupt, G. W., J. Opt. Soc. Am. **42**, 441 (1952).

[16] Haupt, G. W., J. Res. Nat. Bur. Stand. **48**, 414 (1952).

[17] Vandenbelt, J. M., and Spurlock, C. H., J. Opt. Soc. Am. **45**, 967 (1955).

[18] Gridgeman, N. T., Photoelectric Spectrometry Group Bull. **4**, 67 (1951).

[19] Ketelaar, J. A. A., Fahrenfort, J., Haas, C., Brinkman, G. A., Photoelectric Spectrometry Group Bull. **8**, 176 (1955).

[20] Vandenbelt, J. M., J. Opt. Soc. Am. **50**, 24 (1960).

[21] Ringbom, A., Complexation in Analytical Chemistry, (John Wiley & Sons, Inc., New York, N.Y., 1963), p. 294.

[22] Neuss, J. D., and Rieman, W., J. Am. Chem. Soc. **56**, 2238 (1934).

[23] Howard, J. R., Nair, V. S. K., and Nancollas, G. H., Trans. Faraday Soc. **54**, 1034 (1958).

[24] Tong, J., and King, E. L., J. Am. Chem. Soc. **75**, 6180 (1953).

[25] Davies, W. G., and Prue, J. E., Trans. Faraday Soc. **51**, 1045 (1955).

[26] Haight, G. P., Richardson, D. C., and Coburn, N. H., J. Inorg. Chem. **3**, 1777 (1964).

[27] Johnson, E. A., Photoelectric Spectrometry Group Bull. **17**, 505 (1967).

[28] Edisbury, J. R., Photoelectric Spectrometry Group Bull. **1**, 10 (1949).

[29] Edisbury, J. R., Photoelectric Spectrometry Group Bull. **16**, 441 (1965).

[30] Malitson, I., private communication.

[31] Rains, T. C., private communication.

[32] Ewing, G. W., and Parsons, T., Anal. Chem. **20**, 423 (1948).

[33] Swift, T. J., and Connick, R. E., J. Chem. Phys. **37**, 307 (1962).

[34] Thomson, L. C., Trans. Faraday Soc. **42**, 663 (1946).

[35] Hall, H. T., and Eyring, H., J. Am. Chem. Soc. **72**, 782 (1950).

[36] Burke, R. W., and Deardorff, E. R., Nat. Bur. Stand. (U.S.), Tech. Note 544, 153 pages (1970), O. Menis and J. I. Schultz, Eds.

[37] Ciba Chemical and Dye Company, Fair Lawn, New Jersey.

[38] Industrial Chemicals Division, Allied Chemical Corporation, Buffalo, New York.

(Paper 76A5–733)

APPENDIX III

Standard Reference Materials:

Standard Quartz Cuvettes For High Accuracy Spectrophotometry

R. Mavrodineanu

Analytical Chemistry Division
Institute for Materials Research

and

J. W. Lazar

Optical Physics Division
Institute for Basic Standards

National Bureau of Standards
Washington, D.C. 20234

U.S. DEPARTMENT OF COMMERCE, Frederick B. Dent, *Secretary*
NATIONAL BUREAU OF STANDARDS, Richard W. Roberts, *Director*

Issued December 1973

Library of Congress Catalog Number: 73-600200

National Bureau of Standards Special Publication 260-32

Nat. Bur. Stand. (U.S.), Spec. Publ. 260-32, 26 pages (Dec. 1973)

CODEN: XNBSAV

U.S. GOVERNMENT PRINTING OFFICE
WASHINGTON: 1973

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C., 20402
(Order by SD Catalog No. C13.10:260-32). Stock No. SN 003-003-01213-1.Price 55 cents.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

STANDARD REFERENCE MATERIALS:
STANDARD QUARTZ CUVETTES FOR HIGH ACCURACY SPECTROPHOTOMETRY

R. Mavrodineanu

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

and

J. W. Lazar

Optical Physics Division
Institute for Basic Standards
National Bureau of Standards
Washington, D. C. 20234

Accurate knowledge of lightpath and parallelism of cuvettes used in spectrophotometry is one of the indispensable parameters which must be determined when accurate transmittance measurements of liquid materials is considered. A description is given of the design and techniques developed at NBS for the production of quartz cuvettes having a nominal radiation pathlength of 10 mm ± 0.03 mm. For each cuvette the pathlength and parallelism are certified with an uncertainty of ± 0.0005 mm. The method and instrumentation used to determine these parameters is also described in the paper.

Key words: Cuvette, spectrophotometry; lightpath; pathlength; quartz, cuvette; radiation pathlength.

I. INTRODUCTION

True transmittance values can be obtained only when using accurate measuring techniques and by taking into consideration all factors which can affect and distort the data [1]. Since transmittance is the ratio of two radiation flux intensities, it is necessary that the photometric scale of the spectrophotometer used be accurate. Other important conditions which must be satisfied are: wavelength accuracy, use of adequate spectral bandpass, use of collimated radiation, freedom from reflections (interreflection), fluorescence, polarization, light scatter, optical interferences, particulate matter. Surface conditions and the temperature at which the measurements are performed must be defined, and the material subjected to measurements must be homogeneous (freedom of strain) and stable.

When liquid samples are measured the pathlength of the radiation passing through the solution must be accurately known. This last condition is particularly important, not only when accurate transmittance measurements are contemplated, but also when the molar absorptivity of a chemical species is sought. In these cases, an uncertainty of 0.1 mm in a nominal radiation pathlength of 10 mm for instance, will result in an error of 1 percent in the absorbance measurements, and, similarly, an uncertainty of 0.01 mm will result in an error of 0.1 percent absorbance. The object of the work discussed in this paper is to describe the procedures developed and used in the construction of quartz cuvettes having a nominal radiation pathlength of 10 mm ± 0.03 mm, parallelism between the two transparent plates within 0.002 mm, plate flatness of 2 to 3 fringes (mercury green line) and parallelism of these plates within 0.002 mm over the whole length of the plates.

Additional conditions to be met were free from fluorescence and strain, and the use of fused silica of optical grade.

The fulfillment of these specifications would practically eliminate the radiation pathlength error when absorbance measurements are performed at an uncertainty level of 0.1 percent. Such an accuracy seems to be at the limit obtainable by the present state of the art when measurements of absorbance are performed on liquid samples, and include the errors of sample preparation and handling, as well as the instrument uncertainty.

II. EXPERIMENTAL

Several designs were contemplated for constructing quartz cuvettes which would satisfy the rather tight specifications mentioned above. The technique illustrated in figure 1 was finally selected, and was developed in association with E. P. Muth of NBS Optical Shop, who also made all the parts required for the cuvettes discussed in this work. The final assembly was performed by E. I. Klein from NBS Glassblowing Shop.

A rectangular-shaped fused-silica structure 48.0 mm high, 13.5 mm wide and 300 mm long was used to make the body of the cuvettes. Segments approximately 13 mm long were cut from this material and ground and polished, using conventional optical shop techniques, to produce a rectangular segment 10 mm ± 0.03 mm long. The rectangular edges of each segment were finished to a flatness of 1 to 2 fringes (mercury green line), and at one end a small orifice was provided to permit air expansion during the assembly operation.

The length of every segment was checked for accuracy with a standard measuring instrument, illustrated in figure 2a and b, capable of determining this length with an accuracy of ± 0.5 μm.

Two plates made of non-fluorescent fused silica 51 mm long, 16 mm wide and 2 mm thick were ground and polished in a similar manner to a width of 12.5 mm, a thickness of 1.1 mm with a parallelism of 0.002 mm and a flatness of about 2 fringes (mercury green line) as shown in figure 1. These plates were checked for thickness with the instrument illustrated in figure 2a, b and were attached to the rectangular body by careful welding with city gas-oxygen torch using a needle shape flame.

The top of the assembled unit was then cut to produce a cuvette 43 mm high, and a rectangular fused silica block of 6 mm was attached to the open end of the cuvette by fusion. This block was provided with a standard tapered opening with a diameter of 10 mm at the top. A standard-taper Teflon stopper was used to provide tight closure of the cuvette (fig. 1). The cuvette was finished by grinding the two side walls and bevelling the edges. Its nominal dimensions are given in figure 3. The unit was stress-released by proper annealing.

The cuvette was then measured to determine the radiation pathlength and the parallelism of the unit. For this purpose the instrument illustrated in figure 4a and b was used, all measurements being performed at a temperature of 20° ± 0.05 °C. The measurements were made as described below.

The cuvettes were lightly clamped in a fixture which was bolted to the table of a Moore No. 3 Measuring Machine*

---

*The identification of commercial instruments and products is given only to permit reproduction of the work described in this paper. In no instances does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the particular equipment or product is necessarily the best available for the purpose.

and were adjusted until the two plates which define the radiation path length were vertical within the limits of their parallelism. An electronic lever type gage, with a resolution of 0.125 µm was mounted on the spindle. To reach the bottom of the cuvette, a longer lever was fabricated and mounted on the gage. Use of the longer lever required that a correction factor be applied to all measurements which decreased the resolution of the gaging system to 0.312 µm.

As shown in Figure 4b, a combination of gage blocks and end plates were wrung together so that the distance between the inside faces of the end plates was 10.0000 ± 0.0001 mm. The gage blocks were then positioned beneath the center of the spindle and the gage lever was offset from the center of rotation of the spindle so that when the spindle was rotated 180° and the lever contacted each end plate the meter readout of the gaging system was at its null (zero) position.

The machine table was then moved to position the cuvette beneath the spindle. The spindle was lowered until the gage lever was at its first measuring position inside the cuvette. The table was moved until the lever contacted the face of the cuvette and the meter was at its zero position. The spindle was rotated 180° until the gage lever contacted the opposite side of the cuvette. The meter reading multiplied by the correction factor plus the nominal 10 mm path length gave the true pathlength at that point. Measurements were made approximately every 4 mm by lowering the spindle.

Seventeen quartz cuvettes were made and measured at NBS according to the procedures described in this paper, and the values found are presented in table 1.

Table 1. Radiation pathlength expressed in mm and determined on 17 quartz cuvettes made at NBS. The measurements were performed between the internal faces of the transparent plates, at 20°C.

Cuvette No.	Identification of positions at which measurements were made, starting from position 1, 6 mm from top and ending with position 13, 2 mm from bottom of cuvette.											
	1 Top	2	3	4	5	6	7	8	9	10	11	12	13 Bottom
11	9.997_8	9.998_1	9.998_1	9.998_8	9.999_1	9.999_7	9.999_1	10.000_3	10.000_3	10.000_3	10.000_3	10.000_3	10.000_3
12	9.997_8	9.997_2	9.997_5	9.997_5	9.997_5	9.998_1	9.998_4	9.998_1	9.997_8	9.997_5	9.997_8	9.997_8	9.998_4
13	10.008_7	10.000_6	9.999_7	9.999_4	10.000_3	10.000_3	10.001_2	10.001_8	10.002_2	10.002_2	10.001_5	10.001_8	10.002_5
14	9.998_8	9.996_6	9.996_0	9.995_7	9.995_4	9.995_0	9.994_7	9.994_7	9.994_7	9.994_4	9.994_4	9.994_7	9.995_4
15	9.997_8	9.997_8	9.997_8	9.997_2	9.997_2	9.996_6	9.996_9	9.997_2	9.997_2	9.996_9	9.996_9	9.997_2	9.997_8
16	9.999_1	9.998_1	9.997_4	9.997_4	9.998_1	9.998_4	9.999_1	9.999_1	9.999_4	9.999_4	9.999_7	9.999_4	9.999_1
17	9.995_7	9.994_7	9.995_7	9.996_6	9.997_5	9.998_1	9.998_8	9.999_1	9.999_1	9.999_1	9.999_4	9.999_4	9.998_8
18	9.996_6	9.996_0	9.995_6	9.995_0	9.994_4	9.994_4	9.994_7	9.994_7	9.994_7	9.994_4	9.994_7	9.995_0	-
19	9.995_6	9.995_6	9.996_3	9.996_6	9.997_2	9.997_2	9.997_5	9.997_5	9.997_2	9.997_8	9.997_8	9.997_5	9.997_2
20*	9.985_7	9.983_8	9.982_0	9.981_3	9.981_0	9.981_7	9.981_3	9.981_7	9.981_7	9.982_0	9.982_9	9.985_1	9.989_1
21	9.998_4	9.998_4	9.998_8	9.998_8	9.998_9	9.999_1	9.998_9	9.998_4	9.998_4	9.996_9	9.997_8	9.998_1	9.997_5
22	9.999_4	9.998_4	9.997_8	9.998_1	9.998_1	9.998_1	9.997_8	9.997_5	9.997_2	9.997_2	9.997_5	9.997_5	9.998_1
23*	9.991_6	9.990_0	9.989_4	9.989_1	9.989_1	9.989_1	9.988_2	9.986_3	9.985_7	9.986_3	9.985_4	9.987_9	9.989_1
24	9.997_8	9.997_5	9.997_8	9.997_8	9.997_8	9.997_2	9.998_8	9.999_1	9.999_4	9.999_1	9.998_1	9.999_1	9.999_7
25	9.998_8	9.998_4	9.998_1	9.998_8	9.999_1	9.999_1	9.999_4	10.000_3	10.000_3	10.000_6	10.000_6	10.000_6	10.000_3
26	9.998_4	9.998_8	9.998_8	9.999_4	9.999_7	10.000_3	9.999_7	10.000_3	10.000_3	10.000_6	10.000_9	10.000_9	10.001_5
27*	9.996_6	9.996_0	9.996_3	9.996_3	9.996_3	9.996_6	9.996_6	9.996_9	9.996_9	9.997_5	9.998_1	9.998_4	9.999_1

---

* The values obtained for these cuvettes exceed the limits specified for acceptance.

From these data it can be concluded that only three of the 17 cuvettes, or less than 18 percent of the NBS cuvettes, were below the specifications established for acceptance.

The same measurements were performed on 10 quartz cuvettes of commercial origin. The results assembled in table 2 show that only 4 of the 10 cuvettes were acceptable by the standards established in this paper, which indicate a rejection of 60 percent. It must be noted, however, that 9 of 10 cuvettes were within the specifications of the manufacturer which indicate a tolerance of nominal light-path of 10 mm ± 0.01 mm.

III. APPLICATIONS

As a result of these measurements, it was decided to produce at NBS a number of quartz cuvettes, identical to those described in this work, and to certify them for radiation pathlength and parallelism. These cuvettes would then constitute a Standard Reference Material (SRM) in spectrophotometry, and be made available to the public as SRM 932. Each cuvette will have a certificate of calibration of which a reproduction is given here.

Table 2. Radiation pathlength expressed in mm and determined on 10 quartz cuvettes made by a commercial manufacturer. The measurements are performed between the internal faces of the transparent plates, at 20 °C.

Cuvette No.	Identification of positions at which measurements were made, starting from position 1, at 6 mm from top and ending with position 10, 2 mm from bottom of cuvette.									
	1 Top	2	3	4	5	6	7	8	9	10 Bottom
1	10.020_8	10.020_2	10.021_2	10.021_2	10.020_8	10.021_8	10.021_5	10.021_2	10.021_8	10.022_1
2	10.016_2	10.018_7	10.016_5	10.015_2	10.015_9	10.016_5	10.016_8	10.016_8	10.017_1	10.017_1
3*	10.012_1	10.015_6	10.016_9	10.015_6	10.014_8	10.015_9	10.016_9	10.015_1	10.013_3	10.011_0
4	10.002_4	10.003_4	10.004_2	10.003_7	10.003_2	10.003_9	10.004_2	10.004_2	10.003_9	10.002_9
5*	10.014_6	10.016_9	10.017_9	10.015_4	10.013_6	10.015_4	10.012_8	10.013_8	10.012_3	10.012_8
6	10.007_2	10.009_5	10.009_3	10.009_3	10.010_0	10.009_3	10.008_8	10.010_3	10.009_3	10.010_3
7*	10.015_1	10.015_9	10.013_1	10.013_6	10.016_1	10.016_4	10.017_7	10.017_4	10.021_2	10.016_4
8*	10.006_2	10.006_0	10.007_0	10.008_8	10.006_7	10.006_2	10.006_7	10.008_8	10.008_2	10.000_6
9*	10.001_6	10.002_2	10.006_7	10.006_5	10.007_2	10.006_5	10.010_8	10.012_3	10.014_4	10.013_3
10*	10.009_8	10.010_0	10.008_8	10.008_2	10.009_3	10.011_3	10.010_8	10.008_2	10.008_5	10.006_2

---

*The values obtained for these cuvettes exceed the limits specified for acceptance.

National Bureau of Standards

CERTIFICATE

STANDARD REFERENCE MATERIAL 932
QUARTZ CUVETTE FOR SPECTROPHOTOMETRY

R. Mavrodineanu and J. W. Lazar

This Standard Reference Material is intended as a reference source when production of accurate spectrophotometric data on liquid samples is considered. It consists of an all-quartz rectangular cuvette designed to fit the holder of conventional spectrophotometers. It is provided with two optically transparent and parallel windows defining a nominal radiation pathlength of 10 mm ± .03 mm. The inner surfaces of the windows are parallel within .002 mm. The pathlength and parallelism are certified with an uncertainty of ± 0.0005 mm as determined by measurements at positions equally spaced 2 mm from the bottom to within 6 mm of the stopper top. These measurements are made at 20 °C, and the distance between each position is about 4 mm.

Position of Measurement	Radiation Pathlength, mm
Top	9.999
9	9.999
8	10.000
7	9.999
6	10.000
5	9.999
4	9.998
3	9.998
2	9.998
1	9.997
Bottom	9.997

The general shape and nominal dimensions of the cuvette are illustrated in the figure.*

The cuvette must be handled with great care and should be held only by the frosted quartz side windows. When not in use, it should be stored in the container provided for this purpose. Extended exposure to laboratory atmosphere and dusty surroundings is to be avoided.

The overall direction and coordination of the technical measurements leading to certification were performed under the chairmanship of O. Menis and J.A. Simpson.

The technical and support aspects involved in the preparation, certification and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T. W. Mears.

J. Paul Cali, Chief
Office of Standard Reference Materials

Washington, D. C. 20234

March 1, 1973

---

*Note: This figure is identical with figure 3 of this paper.

The cuvette was designed and produced at the National Bureau of Standards by special techniques and from non-fluorescent optical-quality fused silica. The transparent windows are attached to the body of the cuvette by direct fusion, and the unit was strain-released by proper annealing. The overall flatness of the transparent windows is within two fringes (546-nm Hg line), and their parallelism is within two micrometers. The radiation pathlength measurements were performed before and after the assembly of the cuvette using electronic feeler-gage type instruments capable of a resolution of 5 parts in 10^6. SRM 932 is a result of the combined efforts of the Analytical Chemistry Division, Institute for Materials Research, the Optical Physics Division of the Institute for Basic Standards, and the Instrument Shops Division.

E. P. Muth and E. I. Klein designed and assembled the cuvette respectively. The radiation pathlength measurements were performed by E. G. Erber.

SRM 932 was developed to meet the needs expressed by the Standards Committee of the American Association of Clinical Chemists under the chairmanship of George N. Bowers, Jr., M. D., Hartford Hospital, Hartford, Connecticut.

This work was initiated and performed with the approval and encouragement of O. Menis, Section Chief, Analytical Chemistry Division.

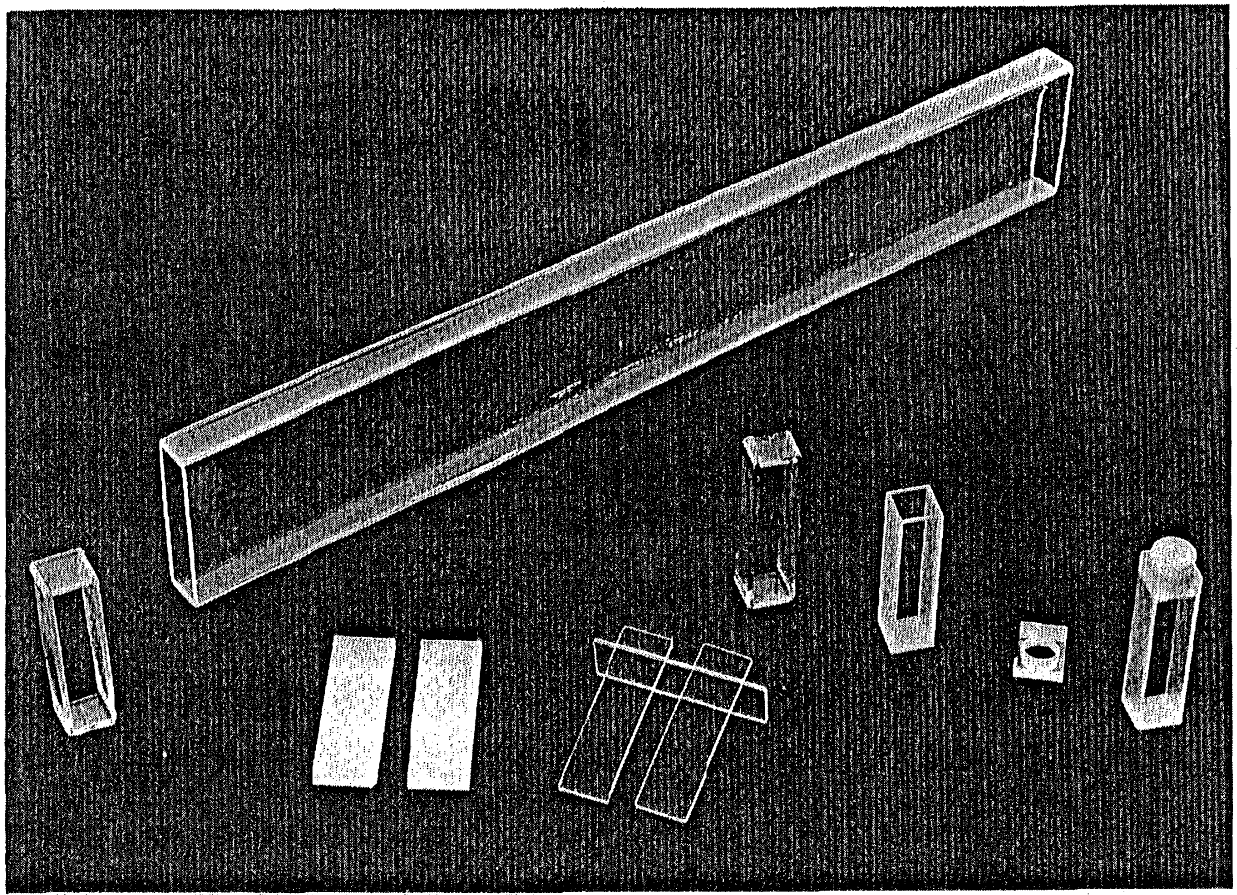


Figure 1. Fused silica material and parts used for constructing the cuvettes for high-accuracy spectrophotometry

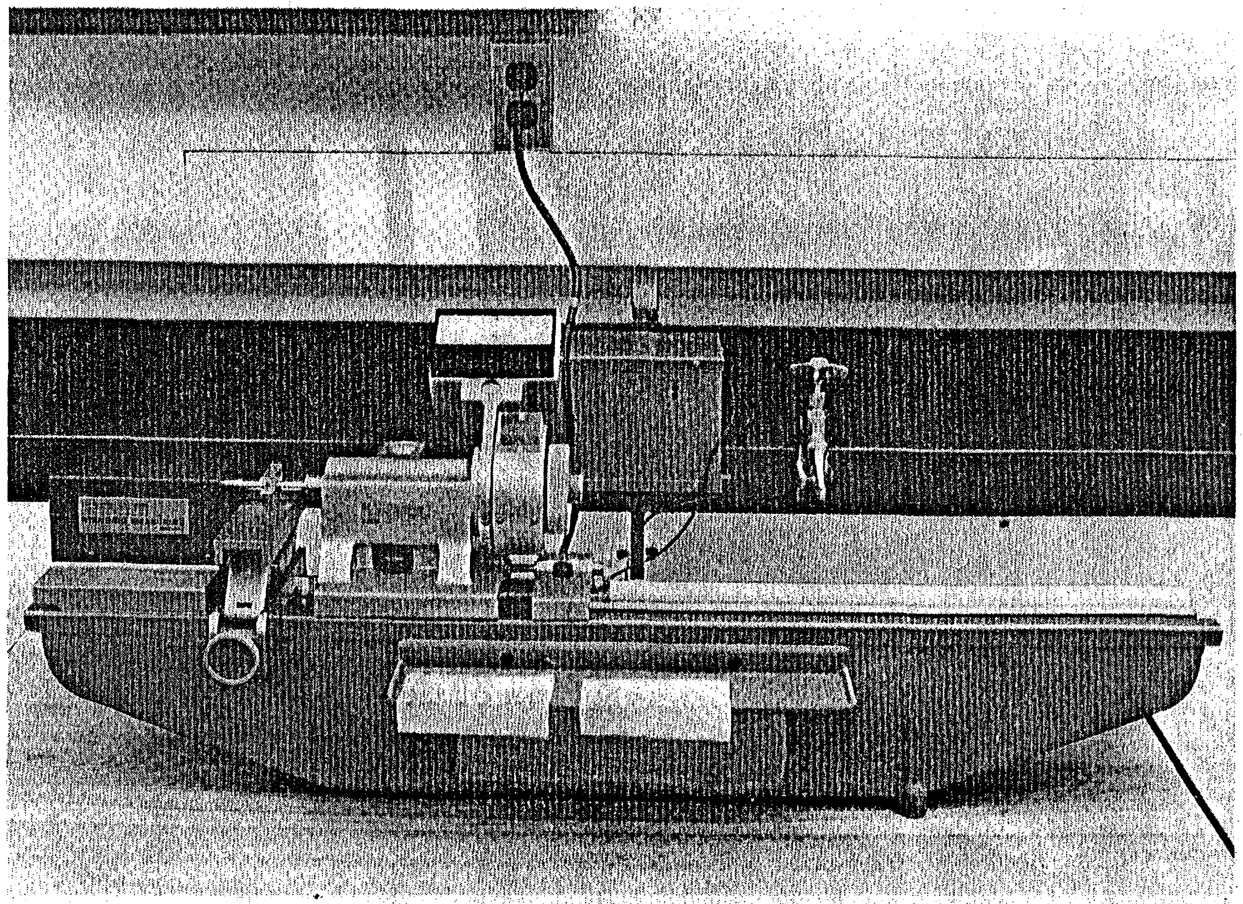


Figure 2a. Standard instrument for measuring the thickness of plates and length of quartz segments.

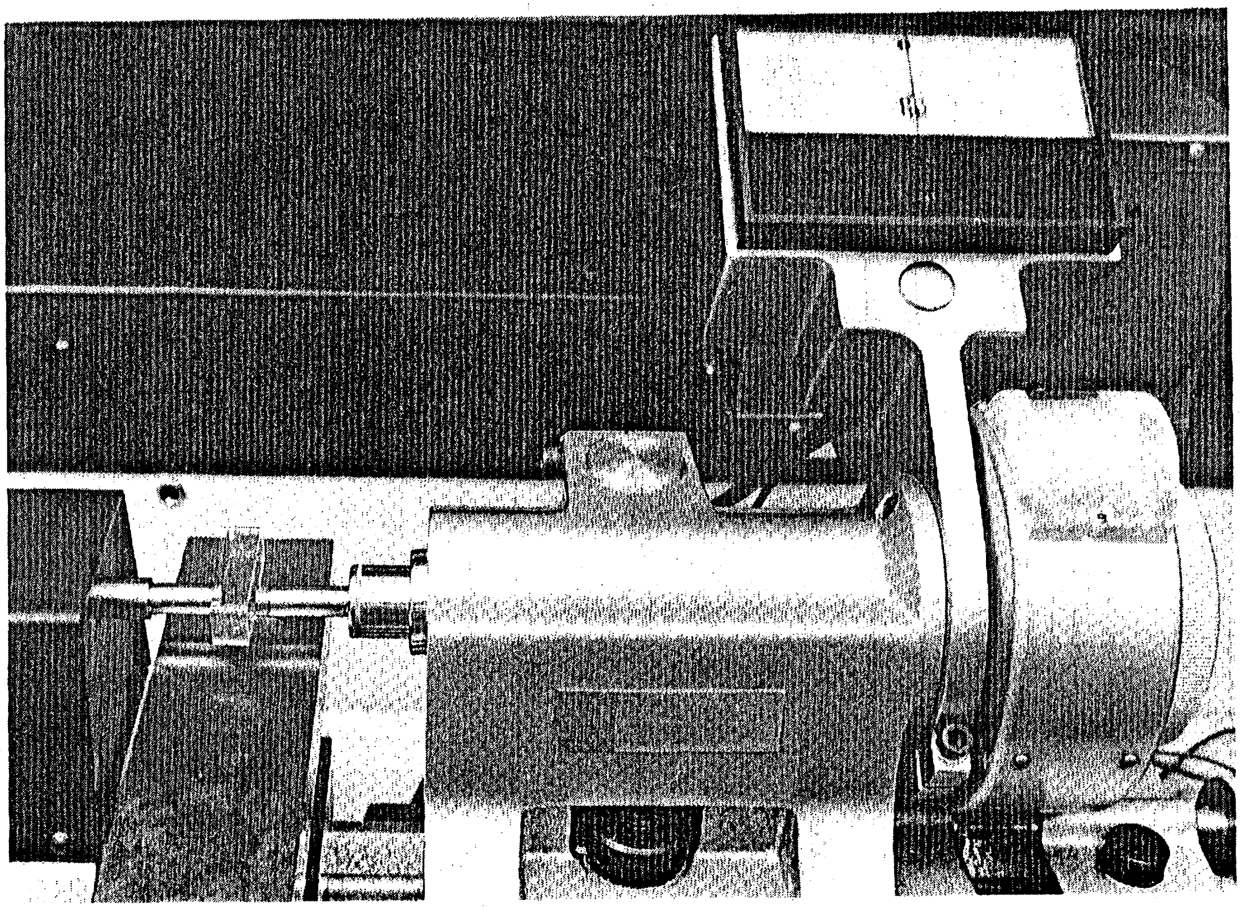


Figure 2b. Close-up of figure 2a.

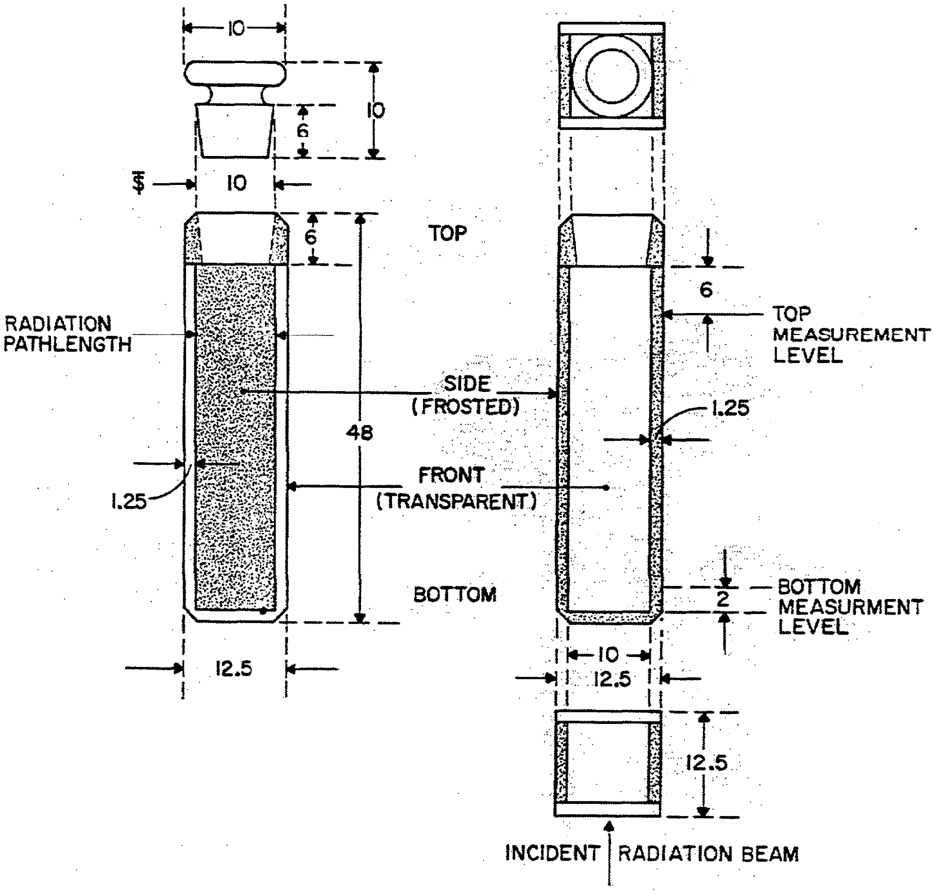


Figure 3. General shape and nominal dimensions (mm) of NBS quartz standard cuvette for spectrophotometry.

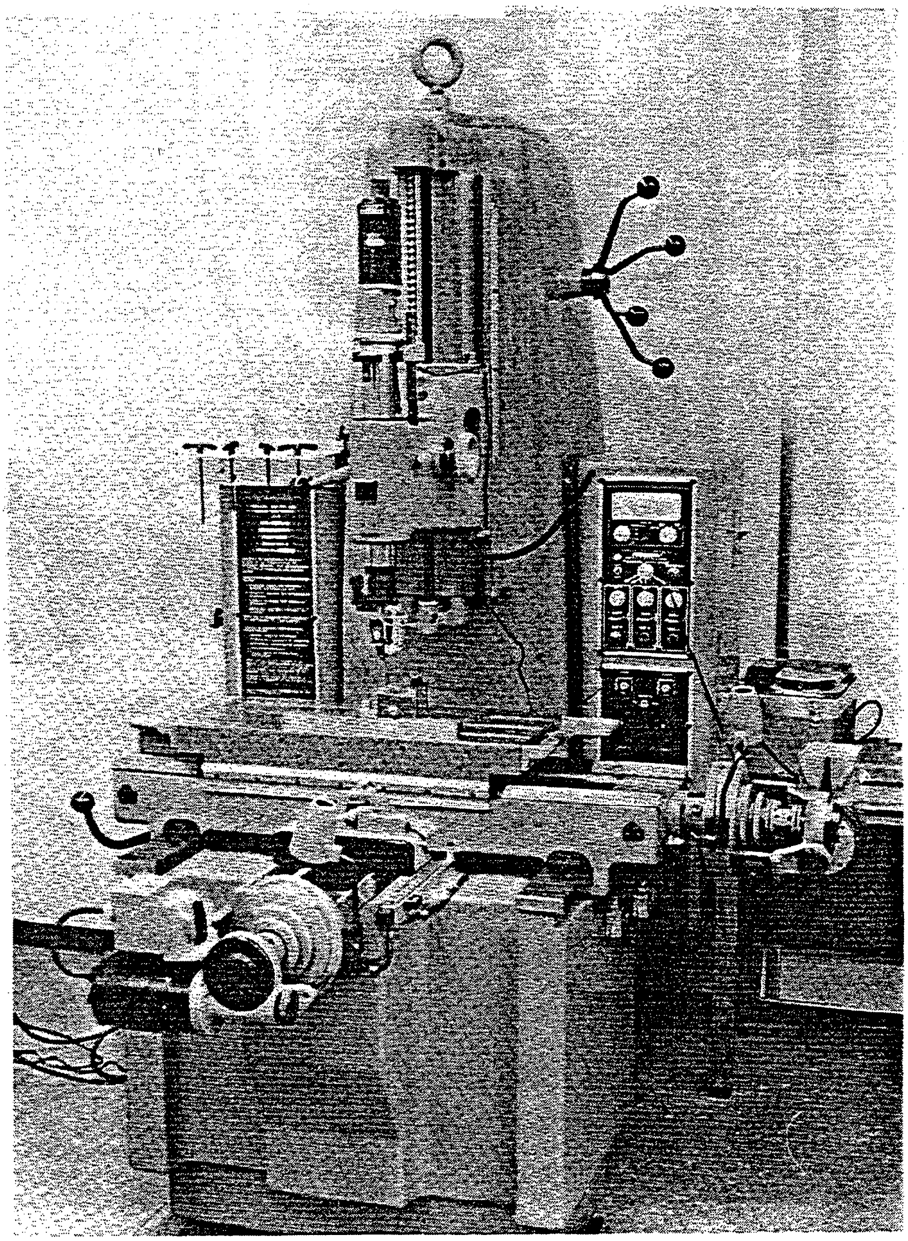


Figure 4a. General view of the standard instrument used to measure the radiation pathlength of cuvettes.

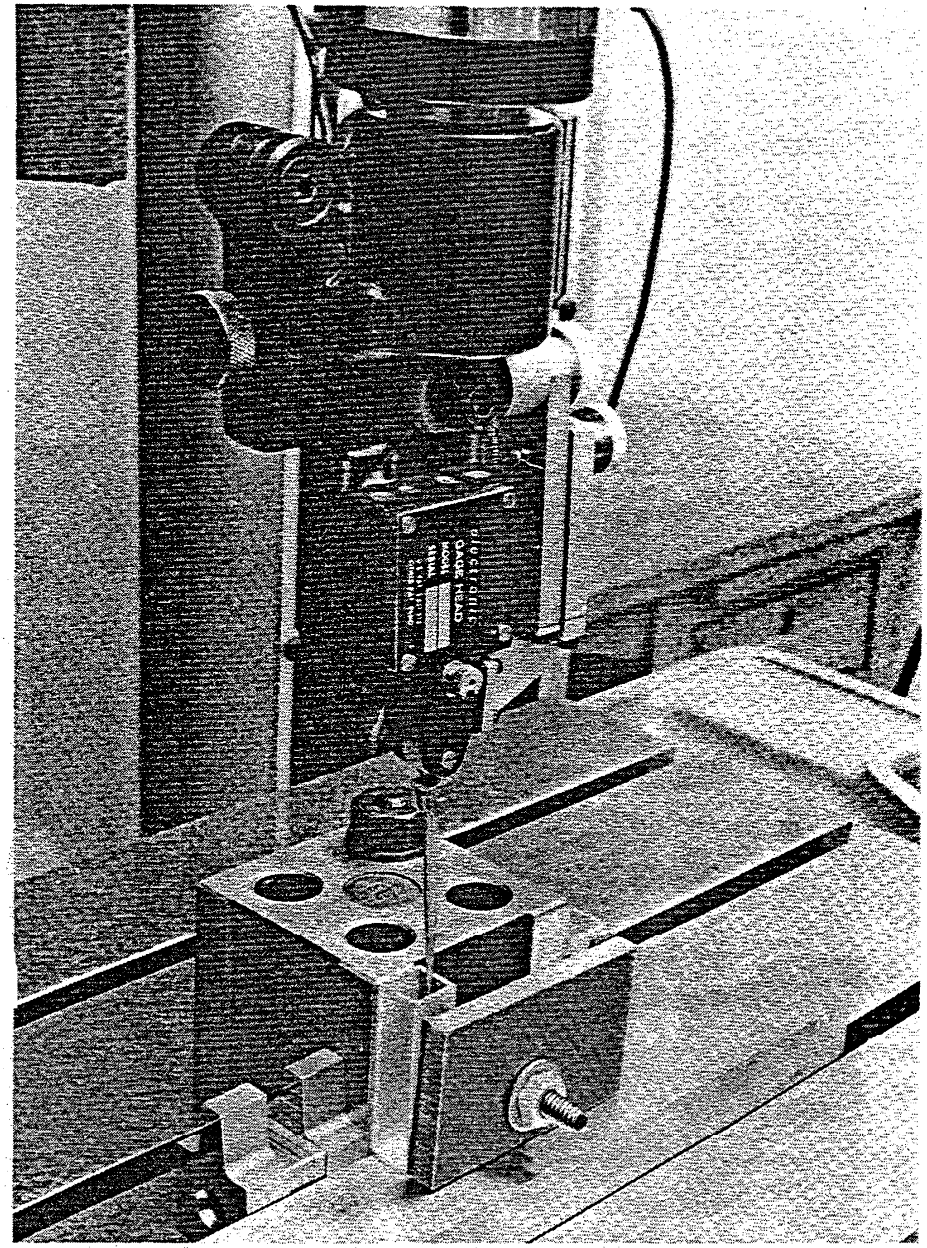


Figure 4b. Close-up of figure 4a.

IV. REFERENCES

[1] Mavrodineanu, R., An Accurate Spectrophotometer for Measuring the Transmittance of Solid and Liquid Materials, J. Res. Nat. Bur. Stand. (U.S.), 76A, 405-425 (1972).

Clarke, F. J. J., High-Accuracy Spectrophotometry, J. Res. Nat. Bur. Stand. (U.S.), 76A, 375-403 (1972).

Mielenz, K. D., Physical Parameters in High-Accuracy Spectrophotometry, J. Res. Nat. Bur. Stand. (U.S.), 76A, 455-467 (1972).

Burke, R. W., Deardorff, E. R. and Menis, O., Liquid Absorbance Standards, J. Res. Nat. Bur. Stand. (U.S.), 76A, 469-482 (1972).

Burnett, R. W., Accurate Measurement of Molar Absorptivity, J. Res. Nat. Bur. Stand. (U.S.), 76A, 483-489 (1972).

☆ U. S. GOVERNMENT PRINTING OFFICE : 1973—542-651/94

APPENDIX IV

JOURNAL OF RESEARCH of the National Bureau of Standards—A. Physics and Chemistry
Vol. 80A, No. 4, July-August 1976

Acidic Potassium Dichromate Solutions as Ultraviolet Absorbance Standards*

R. W. Burke and R. Mavrodineanu

Institute for Materials Research, National Bureau of Standards, Washington, D.C. 20234

(May 18, 1976)

The absorbances of five concentrations of potassium dichromate in 0.001 *M* perchloric acid have been determined at eight wavelengths in the ultraviolet on the National Bureau of Standards Institute for Materials Research high-accuracy spectrophotometer. Four of the wavelengths—235, 257, 313, and 350 nm—correspond to absorbance maxima or minima in the $HCrO_4^-$ spectrum and are useful wavelengths for checking the accuracy of the absorbance scale of narrow bandpass spectrophotometers. Although partial dimerization of $HCrO_4^-$ to $Cr_2O_7^=$ produces small positive deviations from Beer's law at these wavelengths, the apparent absorptivities calculated for each concentration are reproducible to one part in a thousand. The estimated uncertainties in the absorptivity values are ±0.7 percent at 0.1 absorbance (A) and ±0.2 percent near $A=1$. These uncertainties include all known sources of possible systematic error and the 95 percent confidence level for the mean. The remaining four wavelengths used for measurement are near two predicted isosbestic points in the $HCrO_4^-/Cr_2O_7^=$ spectra. The absorptivities at 345 nm are sufficiently independent of concentration that this wavelength can be used for checking absorbance linearity to one part in a thousand over the range $A=0.2$-1.

Key words: Absorbance linearity; accuracy; acidic potassium dichromate solutions; calibration of ultraviolet spectrophotometers; liquid filters; transfer standards; ultraviolet absorbance standards.

I. Introduction

At present, there are no certified standards available from the National Bureau of Standards (NBS) for checking the accuracy of the absorbance scale of spectrophotometers throughout the ultraviolet. The number of analytical applications in this important region of the spectrum, however, continues to increase relatively sharply and the need for such standards is becoming increasingly acute.

The area with perhaps the most serious need for ultraviolet absorbance standards at the moment is clinical chemistry. Workers in this field, for example, frequently use molar absorptivity as an index of purity of their biological standards. Only recently, Burnett [1] [1] has discussed the importance of accurate molar absorptivity measurements in the clinical laboratory. He especially emphasizes the need for spectrophotometric accuracy in clinical enzymology. Not only must the molar absorptivity of the substrate or enzyme-catalyzed reaction product be known accurately but also the individual absorbance measurements on the test sample must be accurate. This dual requirement for accuracy in this instance arises because high-purity and well-characterized enzyme preparations are not yet routinely available for use as standards.

A program has been under way at NBS in the Institute for Materials Research (IMR) since 1969 for the development and issuance of visible and ultraviolet transfer standards for use as Standard Reference Materials (SRM's). Two such standards are currently available: (1) SRM 930, consisting of a set of three neutral Schott NG glass filters and (2) SRM 931, an empirical inorganic solution available at three concentrations in 10-ml ampoules. Neither of these SRM's meets the present needs for absorbance standards in the ultraviolet. The glass filters do not transmit below about 350 nm while the inorganic solution is certified only at one wavelength in the ultraviolet (302 nm) and with an uncertainty of ±1 percent. Transfer standards capable of being certified throughout the range of 200–350 nm and with a smaller uncertainty are being investigated, however. The most promising solid filter at the moment for this purpose is the metal-on-quartz type. Some recent experiences with this filter are discussed in the paper immediately following [2]. Of the chemical or liquid-type filters that have been proposed, the weakly acidic potassium dichromate system is considered the best. A critical evaluation of this system, as well as several other candidate materials, was presented in a previous paper [3].

---

*This work was supported in part by the National Institute of General Medical Sciences.

[1] Figures in brackets indicate the literature references at the end of this paper.

In this paper, we present absorptivity values for five concentrations of potassium dichromate in 0.001 *M* perchloric acid. These concentrations effectively span the absorbance range of $A=0.1-1.5$ when measured in 10 mm cuvettes. The absorptivities were calculated from transmittance measurements at eight wavelengths in the ultraviolet on the IMR high-accuracy spectrophotometer. Four of the wavelengths—235, 257, 313 and 350 nm—correspond to absorbance maxima and minima in the $HCrO_4^-$ spectrum and are useful wavelengths for checking the accuracy of the absorbance scale of narrow bandpass spectrophotometers. Although partial dimerization of $HCrO_4^-$ to $Cr_2O_7^=$ produces small positive deviations from Beer's law at these wavelengths, the apparent absorptivities calculated for each concentration are reproducible to one part in a thousand. The estimated uncertainties in the absorptivity values are ±0.7 percent at 0.1 absorbance (*A*) and ±0.2 percent near $A=1$. These uncertainties include all known sources of possible systematic error and the 95 percent confidence level for the mean. The remaining four wavelengths used for measurement are near two predicted isosbestic points in the $HCrO_4^-/Cr_2O_7^=$ spectra. The absorptivities at 345 nm are sufficiently independent of concentration that this wavelength can be used for checking absorbance linearity to one part in a thousand over the range $A=0.2-1$.

II. Experimental Procedure

The potassium dichromate ($K_2Cr_2O_7$) used in this study was a special lot of analytical reagent grade material obtained from the J. T. Baker Chemical Company.[2] Drying studies performed at 105 °C indicated that the surface moisture of this material was less than 0.01 percent. No measurement of possible occluded water was made. However, a recent paper [4] by Yoshimori and Sakaguchi has shown that $K_2Cr_2O_7$ typically contains 0.01 to 0.02 percent occluded water which can be removed only by crushing and drying at 350 °C.

Coulometric assay of the undried potassium dichromate gave a purity, expressed as an oxidant, of 99.972±0.005 percent at the 95 percent confidence level. Emission spectroscopy indicated that the only significant elemental impurities present were sodium and rubidium. Their concentrations were estimated to be 0.02 and 0.03 percent, respectively.

The concentrations of the $K_2Cr_2O_7$ solutions used throughout this study are expressed on a weight/weight basis. Milligram samples of $K_2Cr_2O_7$ were weighed to the nearest microgram on a microbalance. After dissolution in distilled water shown to be free of reducing impurities (see Discussion), 1 ml of 1 *M* perchloric acid was added and the solutions were diluted approximately to volume in 1-liter volumetric flasks. Each flask was fitted with a double-ribbed Teflon stopper (Kontes Glass Company, Vineland, New Jersey) to prevent evaporation. The weight of each solution was determined on a single-pan top-loading balance having a sensitivity of 0.01 g. NBS-calibrated weights were used to establish the accuracy of the balances. The concentrations of the solutions were then calculated after correcting all weights to vacuum. A solvent blank was prepared by diluting 1 ml of 1 *M* perchloric acid to 1 liter with distilled water.

The Institute for Materials Research high-accuracy spectrophotometer was used for performing the transmittance measurements which, in turn, were converted to absorbance. The design and construction of this instrument have been described in detail by one of us (RM) in reference [5] and will not be repeated here. Similarly, the quartz cuvettes used are also of NBS design and construction and have been described previously [6]. These cuvettes are currently available through the NBS Office of Standard Reference Materials as SRM 932. Each cuvette is certified for path length and parallelism to ±0.0005 mm.

Prior to use the cuvettes were cleaned by soaking in concentrated (18 *M*) sulfuric acid for several hours. In order to minimize the heat of mixing, they were then transferred consecutively to 12, 6 and 3 *M* sulfuric acid before rinsing with distilled water. After rinsing thoroughly, the cuvettes were air-dried under an inverted Petri dish that served as a dust-protective cover.

For sample measurements, five calibrated cuvettes were placed in separate holders in the rotating sample compartment [5] of the IMR high-accuracy spectrophotometer and a reference filter (inconel-on-quartz) was placed in a sixth position. The cuvettes were left in their respective holders for the duration of the experiment. All transmittance/absorbance measurements were made relative to air in a temperature-controlled room at 23.5±0.3 °C. The transfer of solvent blank and sample solutions to and from these cuvettes was made by means of borosilicate, Pasteur-type, disposable pipettes. After being rinsed with the test solution, a final transfer for measurement could be made in 10–15 seconds, after which time the cuvette was immediately stoppered with a snugly-fitting Teflon stopper.

Six sets of solutions having nominal concentrations of 20, 40, 60, 80 and 100 mg $K_2Cr_2O_7$/kg were prepared. Each concentration within a set was measured a minimum of six times at the eight wavelengths of interest. The absorbance, $A=-\log(T_{\mathrm{Sample}}/T_{\mathrm{Blank}})$, was computed for each wavelength and concentration from the average of the six transmittance measurements (T). Absorptivities were then calculated after correcting the absorbances for systematic errors due to cuvette path length, beam geometry and internal multiple reflections. No correction was applied for the reflections discussed by Mielenz and Mavrodineanu [7] from

---

[2] In order to describe materials and experimental procedures adequately, it is occasionally necessary to identify the sources of commercial products by the manufacturer's name. In no instance does such identification imply endorsement by the National Bureau of Standards, nor does it imply that the particular product is necessarily the best available for that purpose.

internal components such as the lenses and slit jaws because these are adequately compensated for by the blank. A detailed account of these corrections and calculations will be given in an NBS 260 Special Publication which is now in preparation.

III. Discussion and Results

The ultraviolet absorbance spectrum of a 40 mg kg^{-1} solution of potassium dichromate in 0.001 *M* perchloric acid is shown in figure 1. The four wavelengths selected for certification of absorptivity of this absorbing system, namely 235, 257, 313 and 350 nm, are also indicated. The maxima and minima are sufficiently broad that serious restrictions are not placed upon instrumental spectral bandwidth requirements. The half bandwidths of the 257 and 350 nm peaks, for example, are approximately 60 nm so that an instrumental spectral bandwidth of 3 nm or less is sufficient for obtaining at least 0.999 of the maximum peak intensities.

The first experiment performed on the IMR high-accuracy spectrophotometer was the determination of the rinse behavior and reproducibility of the transmittance/absorbance measurements of the solvent blank. In the initial studies, measurements were made at 235 nm only, since any problems associated with the transfer of solution were expected to be the greatest at the shortest wavelength used. The results obtained for the five cuvettes employed are shown in figure 2. All exhibit a similar rinse pattern, attaining a minimum and constant absorbance value after 5–6 rinses (the term rinse as used here and throughout the remainder of this paper refers to the exchange of one cuvette volume for a second one). Once the blank measurements were in control, similar absorbance measurements were then made on the first series of $K_2Cr_2O_7$ solutions. Five concentrations having nominal concentrations of 20, 40, 60, 80 and 100 mg $K_2Cr_2O_7$/kg were measured. Table I summarizes the results of these measurements together with the final solvent blank values for 235 nm. The cycle of measurements shown here was then extended to the other seven wave-

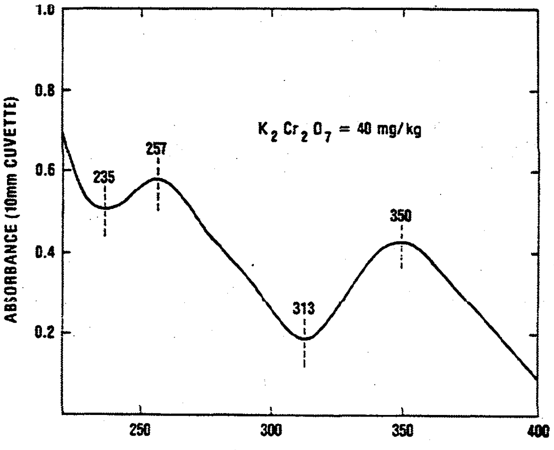


FIGURE 1. *Absorbance spectrum of* $K_2Cr_2O_7$ *in 0.001* M *perchloric acid.*

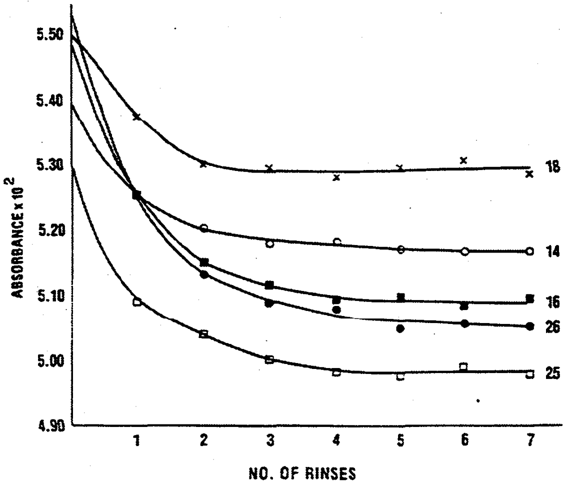


FIGURE 2. *Apparent absorbances of the blank solvent in the five curvettes used—Nos. 14, 16, 18, 25, and 26 ref.* [6].

TABLE I. *Reproducibility of absorbance measurements at 235 nm for a cycle of solvent and sample runs*

Cuvette No.	Solvent	Sample	Sample	Solvent
	After 5 rinses	After 5 rinses	After 7 rinses	After 5 rinses
14	0. 05198	0. 30128	0. 30141	0. 05213
16	. 05099	. 55052	. 55057	. 05093
18	. 05310	. 80066	. 80074	. 05321
25	. 05002	1. 04863	1..04855	. 05022
26	. 05064	1. 30351	1. 30333	. 05065
Ref. filter [a]	. 55913	. 55925	. 55936	. 55922

[a] Inconel-on-quartz.

lengths of interest. In all, six sets of $K_2Cr_2O_7$ solutions were measured. Typically, two volleys consisting of three transmittance/absorbance measurements were made on each of the 30 solutions. The first volley was made after rinsing the cuvettes, initially containing solvent, with five rinses of sample and was repeated again after two additional rinses. Since no systematic increase in absorbance was ever observed when the results of the first volley of measurements were compared to the second, the six measurements were invariably averaged. The absorbances were obtained by subtracting the solvent blank values from the sample readings. After applying appropriate corrections for beam geometry and internal multiple reflections, the corrected absorbances (A_{corr}) were used to calculate the desired absorptivities, using the relationship

$$\text{Absorptivity} = A_{corr}/bc,$$

where b = internal light path in cm and c = concentration of $K_2Cr_2O_7$ solution in g kg^{-1}. The absorptivities computed for the five concentrations of $K_2Cr_2O_7$ solutions used are summarized in table II. The values tabulated were determined from least-squares plots of the experimental values and were subsequently normalized to the concentrations shown. The uncertainties given include all known sources of possible systematic error and the 95 percent (2σ) confidence interval for the mean. The random component of these uncertainties, based on standard deviations computed from residuals resulting from fitting the data to the various concentration levels for each wavelength, is 0.07 percent at the 2σ level.

It is observed that all absorptivities in table II increase with increasing $K_2Cr_2O_7$ concentration. These deviations from Beer's law are produced by the fact that, in weakly acidic media, chromium (VI) ions exist as two distinct absorbing species—$HCrO_4^-$ and its dimerization product, $Cr_2O_7^=$. The equilibrium between these two species may be represented as

$$2HCrO_4^- \overset{K_D}{=} Cr_2O_7^= + H_2O \tag{1}$$

and the corresponding dimerization constant, K_D, is given by

$$K_D = \frac{[Cr_2O_7^=]}{[HCrO_4^-]^2} \tag{2}$$

Although eq. (2) predicts that the formation of $Cr_2O_7^=$ is strictly a quadratic function of $K_2Cr_2O_7$ concentration, the value of $K_D = 32.9$ (mol kg^{-1})$^{-1}$ obtained previously [3] is of such magnitude that the percentage of total chromium present as the $Cr_2O_7^=$ ion is very nearly a linear function of the $K_2Cr_2O_7$ concentration for the range of solutions studied. Calculated values of $HCrO_4^-/Cr_2O_7^=$ speciation in this system are given in table III.

TABLE III. *$HCrO_4^-/Cr_2O_7^=$ speciation in 0.001* M *perchloric acid solutions of $K_2Cr_2O_7$.*

$K_2Cr_2O_7$ Conc., g kg^{-1}	Percent Cr as $HCrO_4^-$	Percent Cr as $Cr_2O_7^=$
0.020	99.10	0.90
.040	98.22	1.78
.060	97.38	2.62
.080	96.56	3.44
.100	95.77	4.23

The direction and magnitude of the expected deviations from Beer's law for the acidic potassium dichromate system can be ascertained when the speciation data in table III are combined with the spectral characteristics of the two chromium (VI) species. Figure 3 shows the relation of the absorbance spectra of the $HCrO_4^-$ and $Cr_2O_7^=$ ions. At wavelengths of 235, 257, 313 and 350 nm, the $Cr_2O_7^=$ ion is shown always to have a larger absorptivity than the $HCrO_4^-$ ion. Hence, the measured absorptivities at these wavelengths should increase with increasing $K_2Cr_2O_7$ concentration.

From the same argument, the data in figure 3 also suggest that there are two wavelengths near 320 and 345 nm where Beer's law is obeyed. In an attempt to determine these isosbestic points experimentally, absorbance measurements were also made on the

TABLE II. *Absorptivities of $K_2C_2O_7$ in 0.001* M *perchloric acid at 23.5 °C*

Absorptivity, kg g^{-1}cm^{-1}

$K_2Cr_2O_7$ Conc., g kg^{-1}	235(1.2)[b]nm	257(0.8)nm	313(0.8)nm	350(0.8)nm	Uncertainty [c]
[a]0.020	12.243	14.248	4.797	10.661	0.034
.040	12.291	14.308	4.804	10.674	[d].022
.060	12.340	14.369	4.811	10.687	[d].020
.080	12.388	14.430	4.818	10.701	[d].020
.100	12.436	14.491	4.825	10.714	[d].019

[a] Nominal concentration; all weights corrected to vacuum.

[b] Wavelength and, ( ), spectral bandwidth.

[c] Includes estimated systematic errors and the 95 percent confidence interval for the mean.

[d] For wavelength of 313 nm, the uncertainty is reduced to half of these values for $K_2Cr_2O_7$ concentrations of 0.040, 0.060, 0.080 and 0.100 g kg^{-1}.

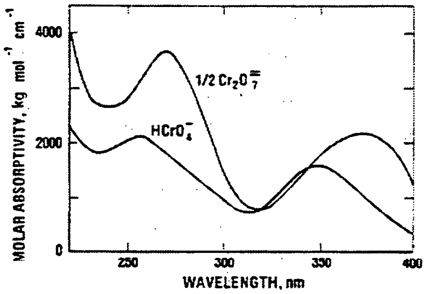


FIGURE 3. *Absorbance spectra of the* $HCrO_4^-$ *ion and its dimerization product,* $Cr_2O_7^=$

same solutions used above at 322, 323, 345 and 346 nm. The corresponding absorptivity values are given in table IV. Although small systematic deviations from Beer's law are still present, the absorptivities calculated for 345 nm are sufficiently constant that the acidic $K_2Cr_2O_7$ system can be used at this wavelength, over the concentration range shown, to check the absorbance linearity of narrow bandpass spectrophotometers to one part in a thousand.

The variation of absorptivity of the acid $K_2Cr_2O_7$ system with temperature is the smallest that we have observed for any liquid filter that we have studied to date. For the four wavelengths recommended for checking the accuracy of the absorbance scale—235, 257, 313, and 350 nm—the absorptivities decrease with increasing temperature. Over the range 20–30 °C, the corrections are, respectively, -0.05, -0.05, -0.02, and -0.05 percent per degree Celsius. The -0.02 percent correction found at 313 nm corresponds closely to the correction predicted from the thermal expansion of the solvent. Until further evaluation can be made of the temperature dependence of 345 nm wavelength recommended for checking absorbance linearity, measurements should be restricted to 23.5 ± 1 °C.

Several considerations were involved in the selection of perchloric acid for acidifying the $K_2Cr_2O_7$ solutions. First, perchloric acid was preferred over the sulfuric acid which has been used traditionally because perchlorate ion, unlike sulfate, has no tendency to form mixed complexes with chromium (VI) species. Secondly, the choice of 0.001 *M* acid rather than 0.01 *M* was based on two factors: (1) the 0.001 *M* acid represented a practical compromise for minimizing conversion of $HCrO_4^-$ to either H_2CrO_4 or $CrO_4^=$ and (2) the lower acid concentration substantially lowered the oxidation potential (~13 percent) of the chromium (VI) ions and hence improved the solution stability of the proposed $K_2Cr_2O_7$ standards.

A final point that needs to be emphasized concerns the quality of the distilled water used for preparing the standard solutions. Acidic potassium dichromate is a potential oxidant and consequently the distilled water used must be shown to be free of reducing impurities in order that the absorptivity data given in this paper be valid. A simple but yet definitive test is outlined in figure 4. In this study a 500-fold dilution of a 100 mg kg^{-1} standard solution of $K_2Cr_2O_7$ was made with the distilled water in question and its absorbance measured at 350 nm. Not only did the measured absorbance agree with the predicted value, thus indicating no reduction of chromium (VI), but also subsequent measurements showed that this $K_2Cr_2O_7$ solution standard should be stable for at least two months provided it is adequately protected against evaporation. In instances where this test shows the distilled water to be suspect, it is recommended that the water be redistilled from alkaline potassium permanganate in order to preoxidize the impurities.

Finally, the general use of solid and liquid transfer standards for calibrating the absorbance scale of a precision commercial spectrophotometer is illustrated in figure 5. The measurements on the glass

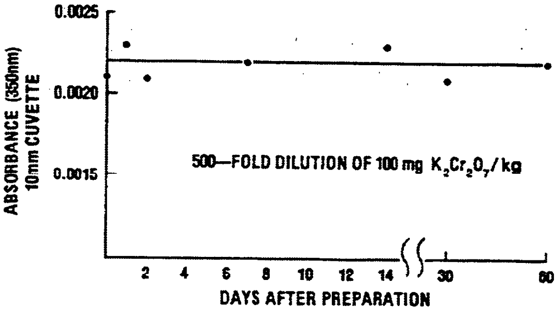


FIGURE 4. *Test of the distilled water for reducing impurities.*

TABLE IV. *Absorptivities of* $K_2Cr_2O_7$ *in 0.001* M *perchloric acid near two predicted isosbestic wavelengths; temperature 23.5 °C*

	Absorptivity, kg g^{-1} cm^{-1}			
$K_2Cr_2O_7$ Conc., g kg^{-1}	322 (0.8)[b] nm	323 (0.8) nm	345 (0.8) nm	346 (0.8) nm
[a] 0. 020	5. 845	6. 065	10. 593	10. 615
. 040	5. 842	6. 062	10. 595	10. 621
. 060	5. 838	6. 059	10. 598	10. 627
. 080	5. 835	6. 056	10. 600	10. 632
. 100	5. 831	6. 053	10. 602	10. 638

[a] Nominal concentration; all weights corrected to vacuum.
[b] Wavelength and, ( ), spectral bandwidth.

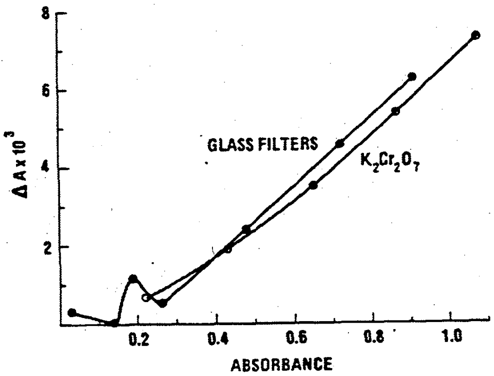


FIGURE 5. *Correction ΔA for the absorbance scale of a precision commercial spectrophotometer.*

filters were performed at 440 nm while the $K_2Cr_2O_7$ measurements were made at 350 nm. At both wavelengths the absorbances measured on the precision spectrophotometer are high and should be corrected by subtracting the appropriate ΔA values.

IV. Conclusion

Following widespread use in the collaborative testing of spectrophotometers for more than 25 years, the absorptivities of the acidic potassium dichromate system have now been determined with a known accuracy from measurements performed directly on the NBS Institute for Materials Research high-accuracy spectrophotometer. Later this year, crystalline $K_2Cr_2O_7$, together with detailed instructions on preparing solutions from it, will be available from NBS through its Office of Standard Reference Materials as an SRM. In conjunction with the calibrated quartz cuvettes previously issued (SRM 932), it is believed that this material will provide a valid check of the accuracy of the absorbance scale of narrow bandpass spectrophotometers in the ultraviolet from 235–350 nm.

The authors gratefully acknowledge the assistance of the following persons at NBS during various phases of this work: K. D. Mielenz, for helpful discussions of fundamental spectrophotometric parameters; G. Marinenko, for performing the coulometric assay; J. A. Norris, for the the emission spectrographic analyses; H. H. Ku, for the statistical analysis of the data; and R. W. Seward, for supplying through the Office of Standard Reference Materials the samples of potassium dichromate.

V. References

[1] Burnett, R. W., J. Res. Nat. Bur. Stand. (U.S.), **76A** (Phys. and Chem.), No. 5, 483–489 (Sept.–Oct. 1972).
[2] Mavrodineanu, R., J. Res. Nat. Bur. Stand. (1976), in press.
[3] Burke, R. W., Deardorff, E. R., and Menis, O., J. Res. Nat. Bur. Stand. (U.S.), **76A** (Phys. and Chem.), No. 5, 469–482 (Sept.–Oct. 1972).
[4] Yoshimori, T. and Sakaguchi, N., Talanta **22**, 233-238 (1975).
[5] Mavrodineanu, R., J. Res. Nat. Bur. Stand. (U.S.), **76A** (Phys. and Chem.), No. 5, 405–425 (Sept.–Oct. 1972).
[6] Mavrodineanu, R., and Lazar, J. W., Nat. Bur. Stand. (U.S.), Spec. Publ. 260-32, 26 pages (Dec. 1973), and in Clin. Chem. **19**, 1053-1057 (1973).
[7] Mielenz, K. D., and Mavrodineanu, R., J. Res. Nat. Bur. Stand. (U.S.), **77A** (Phys. and Chem.), No. 6, 699-703 (Nov.-Dec. 1973).

(Paper 80A4-908)

APPENDIX V

UNITED STATES DEPARTMENT OF COMMERCE • Lewis L. Strauss, *Secretary*
NATIONAL BUREAU OF STANDARDS • A. V. Astin, *Director*

Testing of Glass Volumetric Apparatus

J. C. Hughes

National Bureau of Standards Circular 602
Issued April 1, 1959
[Supersedes Circular 434]

Available as COM 73-10504 from the National Technical Information Services
Springfield, VA 22161

Foreword

This Circular is a revision of NBS Circular 434, by Elmer L. Peffer (deceased) and Grace C. Mulligan. Its purpose is to bring up to date the specifications for glass volumetric apparatus of precision grade, the description of the test methods used, and the directions for submitting apparatus for test.

Specifications for newly developed items have been added, while others have been changed to improve the usefulness of items or to take into consideration new manufacturing methods.

A. V. ASTIN, *Director.*

Contents

Testing of Glass Volumetric Apparatus

J. C. Hughes

This Circular contains specifications and tolerances for glass volumetric apparatus of precision grade. Detailed information is given as to dimensions, graduations, inscriptions, and tolerances for burets, pipets, flasks, cylindrical graduates, and certain kinds of special apparatus.

A description of test methods used and the reports furnished and directions for submitting apparatus are included.

1. Introduction—Purpose and Scope

This Circular presents, to manufacturers and users, information on the design, construction, and verification of glass volumetric apparatus and indicates the facilities available at the Bureau for this verification.

The specifications and tolerances given herein apply to burets, flasks, pipets, and other glass volumetric apparatus of precision grade.

Information is given on the method of reading, the test liquid used, and the methods of test. A description of the tests performed and the reports furnished and directions for submitting apparatus are included.

2. Standard Specifications for Glass Volumetric Apparatus

The primary purpose of these specifications is to define the requisite qualifications for precision apparatus.

The Bureau aims to encourage excellence in quality by cooperating with makers and users of apparatus, and to this end endeavors to assist manufacturers in establishing standards and perfecting their methods. In order that users of standarized apparatus may fully benefit by the facilities of the Bureau, it is necessary for them when purchasing apparatus to be submitted for test to require that the apparatus shall comply with the specifications of the Bureau. By admitting for test only apparatus conforming to these standards, the work of testing is confined to apparatus whose utility is sufficient to justify the labor expended in its accurate calibration. Certain of the specifications, such as those regarding quality of glass and process of annealing before calibration, are largely dependent on the integrity of the maker for their fulfillment. Only by supporting conscientious makers, in giving consideration first to quality and second to cost, can users of standardized apparatus secure a high degree of excellence.

2.1. Types of Apparatus That Will Be Regularly Admitted for Test

Precision grade apparatus of the following types will be accepted for test on written request: transfer pipets and capacity pipets, without subdivisions; burets, measuring pipets, and dilution pipets, with partial or complete subdivisions; volumetric flasks, cylindrical graduates, and specific gravity flasks.

Other types of apparatus should not be submitted without prior arrangement.

2.2. General Specifications[1]

a. Units of Capacity

A *liter* is equal to the volume occupied by the mass of 1 kg of pure water at its maximum density (at a temperature of 4° C, practically).

A *milliliter* (ml),[2] is the one-thousandth part of the liter.

b. Standard Temperature

Although 20° C has been almost universally adopted as the standard temperature for glass volumetric apparatus, many chemists are of the opinion that 25° C more nearly approximates the average laboratory temperature in the United States. The Bureau therefore will calibrate glass volumetric apparatus marked either 20° or 25° C.

c. Material and Annealing

The material should be of best quality glass, transparent and free from striae, surface irregularities, and other defects which may distort the appearance of the liquid surface or the portion of the graduation line seen through the glass. All apparatus should be thoroughly annealed before being graduated.

d. Design and Workmanship

The cross section must be circular and the shape must permit complete emptying and draining and thorough cleaning.

Instruments having a base or foot must stand solidly on a level surface. For all instruments

---

[1] The Bureau reserves the right to reject any apparatus on points affecting its accuracy or utility not covered by these specifications.

[2] The terms "milliliter" and "cubic centimeter" are sometimes used interchangeably. While they are not exactly equal ($1\ \text{ml} = 1.000028\ \text{cm}^3$), the difference is seldom of consequence in volumetric analysis.

except small flasks (below 25 ml) the base shall be of such size that the instrument, empty and without stopper, will stand on a plane inclined 15° to the horizontal. Flasks smaller than 25 ml shall stand on a 10° incline.

Stoppers and stopcocks must be ground or fitted so as to work easily and prevent leakage.

Apparatus which is manifestly fragile or otherwise defective in construction will not be accepted.

The parts on which graduations are placed must be cylindrical for at least 1 cm on each side of every mark (except on certain small flasks and special apparatus), but elsewhere may be enlarged to provide the desired capacities in convenient lengths.

Two scales are not permitted on the same piece of apparatus. For example, apparatus should not be graduated in both fluid ounces and milliliters. In the case of two units, one of which is an exact multiple of the other, such, for example, as drams and fluid ounces, there is no objection to having the 8-dr line, 16-dr line, etc., marked respectively, 1 fl oz, 2 fl oz, etc., provided that the two series of numbers are placed on opposite sides of the apparatus and the value of each subdivision is suitably indicated.

e. Graduation Lines

Graduation lines shall be fine, clean, permanent, continuous, and of uniform width, perpendicular to the axis and parallel to the base of the apparatus. Line width should not exceed 0.3 mm for subdivided apparatus and 0.4 mm for single-line apparatus.

All graduations must extend at least halfway around; and on subdivided apparatus at least every 10th mark, and on undivided apparatus, all marks must extend completely around the circumference. Subdivided apparatus must be provided with a sufficient number of lines of suitable length to facilitate reading.

The clear space between two adjacent marks must be not less than 1 mm wide. The spacing of marks on subdivided apparatus must show no apparent irregularities, and sufficient divisions must be numbered to readily indicate the intended capacity of any interval.

f. Inscriptions

Every instrument must bear in permanent legible characters the capacity, the temperature at which it is to be used, the method of use—that is, whether to contain or to deliver—and on instruments which deliver through an outflow nozzle, the time required to empty the total nominal capacity with unrestricted outflow. Etching is preferred for inscriptions, although engraved or fused marking is acceptable provided such marking is neat and clear. Grit-blasted serial numbers will be permitted with the same provision.

Every instrument must bear the name or trademark of the maker. Every instrument must bear a permanent identification number, and detachable parts, such as stoppers, stopcocks, etc., belonging thereto, if not interchangeably ground, must bear the same number. Interchangeable ground-glass parts shall be marked on both members with the proper standard taper symbol and the size designation, in accordance with Commercial Standard CS 21–57.

Figure 1 illustrates several arrangements of designating marks that are considered suitable. Marks may be placed elsewhere on apparatus if they are easily readable and do not interfere with the proper use of the apparatus.

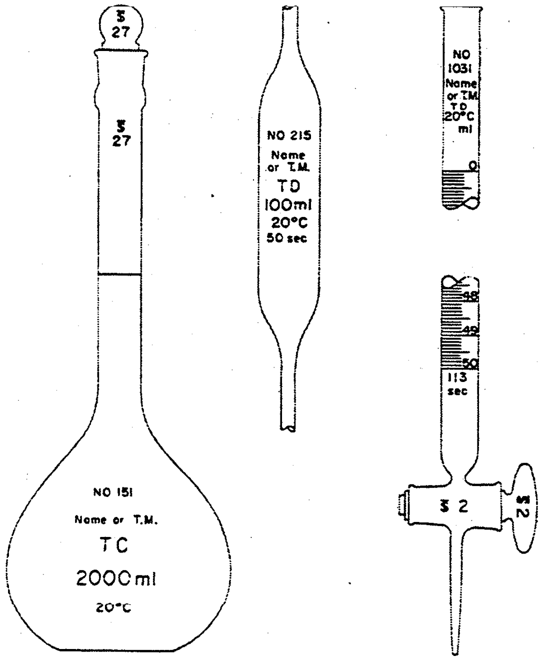


FIGURE 1. *Examples of inscriptions suitable for volumetric apparatus.*

2.3. Special Requirements

a. Flasks

The limiting dimensions for precision-grade volumetric flasks are given in table 1. For flasks smaller than 5-ml capacity, the limits on location of the graduation line will be the same as shown for a 5-ml flask.

Stopper sizes should be so chosen that the smallest diameter of the ground zone in the flask neck is approximately equal to the inside diameter at the graduation line. The inside diameter of the neck at the graduation line should never exceed the smallest diameter of the ground zone by more than 1.5 mm. The shoulder at the base of the ground zone should be smoothly rounded to permit complete drainage.

TABLE 1. *Limiting dimensions for volumetric flasks*

(In millimeters)

Capacity	Inside diameter of neck at graduation line		Minimum distance between graduation line and:		
			Top of flask		Bulb [a]
	Minimum	Maximum	Stoppered type	Unstoppered type	
ml					
5	6	7	22	22	5
10	6	8	28	28	7
25	6	8	35	30	10
50	6	10	40	30	10
100	8	12	40	30	10
200	9	14	55	45	10
250	10	15	55	45	10
500	12	18	60	60	20
1,000	14	20	70	60	20
2,000	18	25	70	60	20

[a] The distance between the graduation line and the bulb is measured to the point where the neck begins to expand into the bulb (on outside).

A flask may be graduated both *to contain* and *to deliver*, provided the intention of the different marks is clearly indicated and provided the distance between the two marks is not less than 1 mm.

b. Cylindrical Graduates

Cylinders may be graduated either to contain or to deliver, but a scale numbered both up and down the length of the graduate is not permitted, as it is obvious that the same graduate cannot be correct both to contain and to deliver.

The relation of the height to the diameter must be such that the graduation marks are not less than 1 mm apart, and also that the graduated height is at least five times the inside diameter. In the case of the 10-ml cylinder subdivided to 0.1 ml, and the 25-ml cylinder subdivided to 0.2 ml, it will be found necessary to make the graduated height considerably more than five times the inside diameter in order to give a separation of 1 mm to the graduation marks. To avoid excessive height, the subdivisions on 10-ml, 25-ml, and 50-ml cylinders may be 0.2 ml, 0.5 ml, and 1.0 ml, respectively.

Subdivision lines shall be omitted between the base and the first numbered line. This will eliminate readings near the base which are difficult and not always accurate. (See fig. 2.)

The numbers indicating the capacity of the graduate at its different points should be placed immediately above the marks to which they refer.

Method of use—Graduates that are to be used dry to receive and measure liquids should be calibrated to contain.

Graduates that are to be used to pour water into other measures, and those which are to be used wet to contain water from other measures, should be calibrated to deliver. For example, a graduate that is to be used in testing milk bottles, either by pouring water from the graduate into the bottles or from the bottles into the graduate without drying the graduate between bottles, should be calibrated to deliver. After having been wet the graduate will, on successive fillings and emptyings, deliver the same quantity that is poured into it.

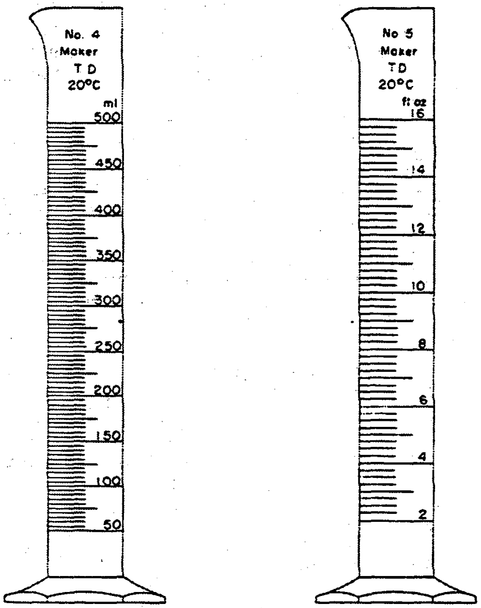


FIGURE 2. *Examples of cylindrical graduates.*

In ordering graduates, the purchaser should consider the use to which they are to be put and should specify accordingly whether they should be calibrated to contain or to deliver.

Basis of graduation—Graduates in customary units should be graduated in accordance with the following relations:

60 minims=1 fl dr
8 fl dr=1 fl oz
32 fl oz=1 liq qt
4 liq qt=1 U. S. gal
1 U. S. gal=231 in.^{3}

For conversion to the metric system, the relation is:

1 fl oz=29.5729 ml

TABLE 2. *Limits of distance from highest graduation to top of graduate*

Total nominal capacity (customary units)	Distance of highest graduation from top		Total nominal capacity (metric units)
	Minimum	Maximum	
Minims	*cm*	*cm*	*ml*
60	2	4	5
120	2	4	10
fl oz			
1	2	5	25
2	3	5	50
4	3	6	100
8	4	8	250
16	5	10	500
32	5	10	1,000

TABLE 3. *Minimum neck diameter of cylinders with stoppers*

Capacity ml	5	10	25	50	100	250	500	1,000
Minimum diameter of neck mm	6	7	8	9	10	12	15	18

c. Transfer Pipets

Pipets for delivering a single volume are designated "transfer" pipets.

The suction tube of each transfer pipet must be at least 16 cm long, and the delivery tube must be not less than 3 cm nor more than 25 cm long. The top of the suction tube must be finished with a smooth plane surface, at right angles to the axis.

The outside diameter of the suction and delivery tubes of transfer pipets, exclusive of the tip, must be not less than 5 mm. Limits of inside diameter at the capacity mark are given in table 4.

The capacity mark on transfer pipets must be not more than 6 cm from the bulb.

The outlet of any transfer pipet must be of such size that the free outflow shall last not more than 1 min and not less than the times shown in table 5 for the respective sizes.

TABLE 4. *Inside diameter of transfer pipets at capacity mark*

Capacity of pipets (in milliliters) up to and including	25	50	200
Diameter (in millimeters): Max	4	5	6
Min	2	2	2

TABLE 5. *Minimum delivery times for transfer pipets*

Capacity (in milliliters) up to and including	5	10	50	100	200
Outflow time (in seconds)	15	20	30	40	50

d. Burets and Measuring Pipets

Only those burets emptying through a nozzle permanently attached at the bottom are accepted for test.

Side tubes, unless provided with stopcocks, are not permitted on burets.

So-called Schellbach burets—that is, those having a milk-glass background with a colored center line—will not be accepted for test on account of possible errors resulting from parallax.

The distance between the extreme graduations must not exceed 70 cm on burets nor 35 cm on measuring pipets.

The rate of outflow of burets and measuring pipets must be restricted by the size of the tip, and for the graduated length the time of free outflow must be not more than 3 min nor less than the times shown in table 6.

The upper end of any measuring pipet must be not less than 10 cm from the uppermost mark and the lower end not less than 4 cm from the lowest mark.

On 50- and 100-ml burets, the highest graduation mark should be not less than 4 cm nor more than 10 cm from the upper end of the buret.

TABLE 6. *Minimum delivery times for burets and measuring pipets*

Length graduated	Time of outflow not less than—	Length graduated	Time of outflow not less than—
cm	*sec*	*cm*	*sec*
15	30	45	80
20	35	50	90
25	40	55	105
30	50	60	120
35	60	65	140
40	70	70	160

On burets having a capacity of 25 ml or less this distance should be not less than 3 cm nor more than 6 cm. (These requirements do not apply to burets where the "zero" is at the end of an overflow tip.)

e. Buret and Pipet Tips

Buret and pipet tips should be made with a gradual taper of from 2 to 3 cm, the taper at the extreme end being slight.

A sudden contraction at the orifice is not permitted, and the end of the tip must be ground perpendicular to the axis of the tube. The outside edge should be beveled slightly and all ground surfaces polished.

In order to facilitate the removal of drops and to avoid splashing, the tip of a buret may be bent slightly.

Approved forms of tips for burets, measuring pipets, and transfer pipets are shown in figure 3.

2.4. Special Apparatus

a. Giles Flasks

The permissible error in the volume at the first mark shall be the tolerance allowed for a flask of that capacity. The permissible error in the volume at the second mark shall be the tolerance at the first mark plus the tolerance for a flask of capacity equal to the difference between the two marks, provided, however, that the error in the volume between the two marks shall not exceed the permissible error in the volume indicated by the first mark.

At the capacity mark the inside diameter of the neck shall be within the limits given in table 7.

The neck shall be cylindrical on each side of every graduation mark for at least the distances given in table 8.

On account of the bulb in the neck of a Giles flask it is more unstable or topheavy than a flask of the ordinary type. For that reason it has been thought advisable to allow a somewhat shorter minimum length for the cylindrical portion of the neck, on each side of the graduation mark, than is allowed on ordinary flasks. In other respects the same general specifications apply to the Giles flasks as to other volumetric apparatus.

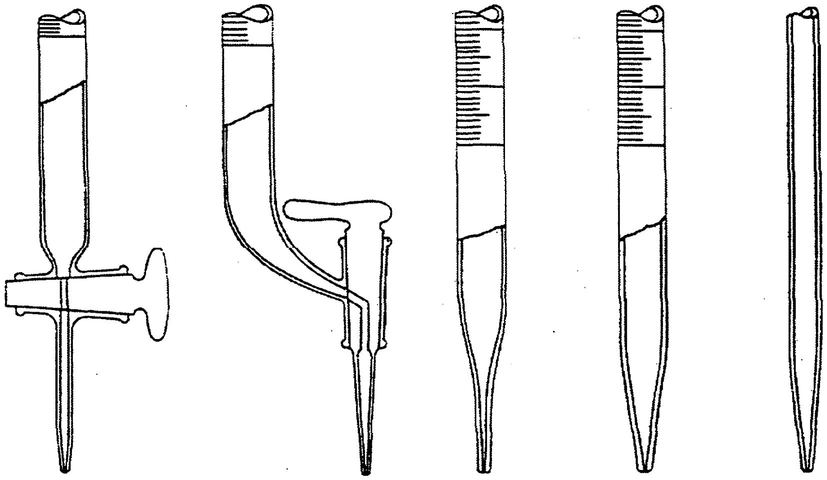


FIGURE 3. *Types of buret and pipet tips.*

TABLE 7. *Neck diameters of Giles flasks*

Capacity	Inside diameter of neck	
	Minimum	Maximum
ml	*mm*	*mm*
25 to 27.5	6	9
50 to 55	6	11
100 to 110	8	13
200 to 220	9	15
500 to 550	12	18
1,000 to 1,100	14	20
2,000 to 2,200	18	25

TABLE 8. *Minimum length of cylindrical portions of neck on Giles flask*

Capacity	Above upper graduation mark	Between upper graduation mark and bulb	Between lower graduation mark and bulb	Below lower graduation mark
ml	*cm*	*cm*	*cm*	*cm*
25 to 27.5	3	0.8	1	1
50 to 55	3	.8	1	1
100 to 110	3	.8	1	1
200 to 220	4	.8	1.5	2
500 to 550	4	1.0	1.5	2
1,000 to 1,100	5	1.0	1.5	2
2,000 to 2,200	5	1.0	1.5	2

b. Specific Gravity Flasks

Material and annealing—The material from which the flasks are made shall be glass of the best quality, transparent, and free from striae. It shall adequately resist chemical action and have small thermal hysteresis. The flasks shall be thoroughly annealed before being graduated. They shall be of sufficient thickness to insure reasonable resistance to breakage.

Design—The cross section of the flask shall be circular, and the shape and dimensions shall conform to the diagram shown in figure 4. This design is intended to insure complete drainage of the flask on emptying and stability of standing on a level surface, as well as accuracy and precision of reading. There shall be a space of at least 1 cm between the highest graduation mark and the lowest point of the grinding for the glass stopper.

Capacity—The flask should contain approximately 250 ml when filled to the zero graduation mark.

Graduations—The neck shall be graduated from 0 to 1 ml and from 18 to 24 ml into 0.1-ml divisions. There shall be two 0.1-ml graduations below the 0 and two above the 1-ml graduation.

Standard temperature—The flasks shall be standard at 20° C. The indicated specific gravities will then be at 20° referred to water at 4° as unity—that is, density at 20° in grams per milliliter.

Inscriptions—Each flask shall bear a permanent identification number and the stopper, if not interchangeably ground, shall bear the same number. The standard temperature shall be indicated and the unit of capacity shall be shown by the letters "ml" placed above the highest graduation mark.

Tolerance—The error of any indicated capacity shall be not greater than 0.05 ml.

Interpretation of the specification—The foregoing specification is intended to represent the most desirable form of specific-gravity flask for use in testing cements. Variations of a few millimeters in such dimensions as total height of flask, diameter of base, etc., are to be expected and will not be considered sufficient cause for rejection. The requirements in regard to tolerance, inscriptions, and the length, spacing, and uniformity of graduations will, however, be rigidly observed.

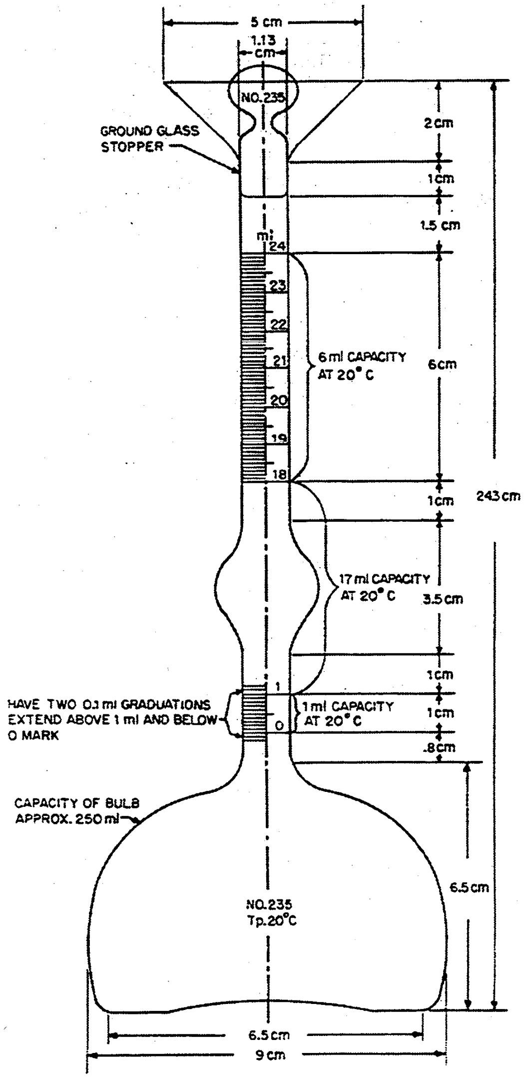


FIGURE 4. *Specific-gravity flask.*

c. Sugar-Testing Flasks (Bates)

The flask shall have a height of 130 mm, and the neck shall be 70 mm in length; a tolerance of ±5 mm is allowed for each. The internal diameter shall be not less than 11.5 mm and not more than 12.5 mm. The upper end of the neck shall be flared, and the graduation mark shall be not less than 30 mm from the upper end and 15 mm from the lower end of the neck. The flask shall be standardized to contain 100 ml at 20° C with a tolerance of 0.08 ml.

d. Babcock Milk Test Apparatus

Because most of the States now require that all Babcock glassware used in the State be approved by officials of that State, the National Bureau of Standards no longer accepts Babcock bottles and pipets for calibration, except when specifically requested by a State to settle a dispute. Specifications for Babcock glassware may be found in official procedures of the Association of Official Agricultural Chemists and certain dairy associations.

e. Dilution (Hemacytometer) Pipets

The pipets should be of good quality glass, free from bubbles and other defects, and sufficiently strong to withstand normal usage. Tubing with milk-glass backing is permissible. The capillary should be of uniform diameter, except that the tip of a white cell pipet may be tapered slightly. The inside diameter at the tip should be between 0.3 and 0.5 mm. On all pipets the tip should be ground smooth and at right angles to the axis of the pipet, and beveled so that the external diameter at the end does not exceed 2.0 mm. All ground surfaces should be polished to restore transparency.

The bulb or mixing chamber should contain a small, nonspherical glass bead to aid in mixing the blood and diluting fluid. Pipets for red blood cells may be designated by a red bead, by a red stripe running parallel to the axis, or both. White cell pipets should have white or clear beads.

The bulb should be so shaped that it can be filled without entrapping air bubbles. The capacity of the bulb is usually about 0.8 to 1.2 ml for the red cell pipet, and 0.2 to 0.4 ml for the white cell pipet.

The pipet should be so graduated as to give dilution ratios of 1 to 10, or 1 to 100, and may be further graduated to give ratios of 0.1, 0.2, 0.5, etc., to 10, and to 100; that is, the interval 0 to 1 may be subdivided to 0.1 or 0.5. The subdivisions, when present, should be uniformly spaced along the capillary tube. The graduations at 0.5, 1.0, 11, and 101 should be numbered.

All numbered graduation lines should extend at least halfway around the pipet, diametrically opposite to the milk-glass backing. Etched lines are preferred, but engraved or fused lines are acceptable provided they meet the requirements of paragraph 2.2.e. The "1" line shall be located not less than 2 mm nor more than 5 mm below the bulb, and the "11" (or "101") line shall be not less than 2 mm nor more than 4 mm above the bulb. Measurement is from the points where the capillaries begin to expand into the bulb.

Dilution pipet tolerances—If V represents the volume between the marks immediately above and below the bulb, v_1 represents the volume of the capillary between the tip and the line marked "1", and v_2 represents the volume of the capillary between the tip and the line marked "0.5", the tolerances may be expressed as follows:

Red cell pipets—nominal ratio 100:1,
  V/v_1 must be not less than 95 and not more than 105
  V/v_2 must be not less than 190 and not more than 210.
White cell pipets—nominal ratio 10:1,
  V/v_1 must be not less than 9.65 and not more than 10.35
  V/v_2 must be not less than 19.30 and not more than 20.70.

In an effort to give more exact information as to accuracy, certain manufacturers have marked dilution pipets with a so-called "correction factor". This is usually written simply "+2", "−1", etc. Because of the possibility that such a correction will not be applied as the manufacturer intended, and because mass-production test methods cannot always be relied upon to fix the corrections within 1 percent, the Bureau does not recommend this practice. In those cases where greater accuracy is required than is provided by the application of the prescribed tolerances (±5% for red cell pipets and ±3.5% for white cell pipets), it is suggested that the purchaser specify the desired limits of error. This would provide the needed accuracy without the application of corrections.

The Bureau does not accept for test pipets bearing a "correction factor" except in very special cases and only in small quantities.

f. Sahli Hemoglobin Pipets

The capacity tolerance for Sahli pipets is ±2 percent of the nominal volume.

g. Immunity Unit Cylinders and Pipets

These instruments were designed by the Hygienic Laboratory of the U. S. Public Health Service (USPHS) for use in the measurement of toxin and serum dilutions. Specifications may be found in USPHS Hygienic Laboratory Bulletin 21. The instruments are made to conform in general with the specifications contained in this Circular, but with certain modifications necessary to adapt them to the specific application.

The pipets are of three types: (1) Capacity or "wash-out" pipets, calibrated to contain the nominal volume; (2) delivery pipets, calibrated to deliver the nominal volume when the residue in the tip after free outflow has ceased is blown out and added to the initial delivery; (3) graduated delivery pipets, called Ehrlich pipets, calibrated to deliver the contents in the same manner as transfer pipets.

Tolerances for the first two types of pipets named are the same for ordinary transfer pipets; for the Ehrlich delivery pipets, the tolerance is 0.01 ml. For the cylinders, the tolerances are: For capacities less than and including 50 ml—0.05 ml; for capacities over 50 ml and including 100 ml—0.08 ml.

h. Special-Purpose Volumetric Flasks

Certain types of special-purpose volumetric flasks such as the Engler viscosity flask and the Kohlrausch and Stift sugar flasks, while not in conformity with all the special requirements for volumetric flasks listed in section 2.3.a because of their specific applications, will be calibrated and marked with the precision stamp if they are in satisfactory conformity with the general specifications in section 2.2 and the errors in capacity do not exceed the tolerances for volumetric flasks given in section 2.5.

i. Other Special Apparatus

Other types of special-purpose instruments may be calibrated and marked with the precision stamp if they conform with the general specifications (section 2.2) and the errors in capacity do not exceed the applicable tolerances in section 2.5.

Permanent identification numbers are required on all glass volumetric apparatus submitted for certification.

Instruments obviously not capable of precision measurements, as for example, centrifuge tubes, are not accepted because the time expended in their calibration is not warranted by the degree of accuracy required in their use.

2.5. Tolerances

a. Flasks

The capacity tolerances for flasks are given in table 9.

TABLE 9. *Capacity tolerances for volumetric flasks*

Capacity (in milliliters) less than and including—	Limit of error	
	If to contain—	If to deliver—
	ml	*ml*
1	0.01	------------
3	.015	------------
5	.02	------------
10	.02	0.04
25	.03	.05
50	.05	.10
100	.08	.15
200	.10	.20
300	.12	.25
500	.15	.30
1,000	.30	.50
2,000	.50	1.00
Above 2,000	1 part in 4,000	1 part in 2,000

b. Transfer Pipets

The capacity tolerances for transfer pipets are given in table 10. These tolerances are applicable to pipets of similar design made *to contain* the nominal volumes.

c. Burets and Measuring Pipets

The capacity tolerances for burets and measuring pipets are given in table 11.

Table 10. *Capacity tolerances for transfer pipets*

Capacity (in milliliters) less than and including—	Limit of error
	ml
2	0.006
5	.01
10	.02
30	.03
50	.05
100	.08
200	.10

Table 11. *Capacity tolerances for burets and measuring pipets*

Capacity (in milliliters) of total graduated portion less than and including—	Limit of error of total or partial capacity	
	Burets	Measuring pipets
	ml	*ml*
2	--------	0.01
5	0.01	.02
10	.02	.03
30	.03	.05
50	.05	.08
100	.10	.15

d. Cylindrical Graduates

The capacity tolerances for cylindrical graduates are given in table 12.

e. Delivery Time

The actual delivery time of any instrument must be within the limits prescribed in section 2.3, and the error in the marked delivery time must not exceed the limits given in table 13.

Table 12. *Capacity tolerances for cylindrical graduates*

Metric units				Customary units			
Capacity of total graduated portion less than and including—	Maximum diameter	Limit of error of total or partial capacity		Capacity of total graduated portion less than and including—	Maximum diameter	Limit of error of total or partial capacity	
		If to contain—	If to deliver—			If to contain—	If to deliver—
ml	*mm*	*ml*	*ml*	*Minims*	*in.*	*Minims*	*Minims*
5	11	0.05	0.06	60	3/8	0.6	0.7
10	14	.08	.10	120	1/2	1.0	1.3
25	19	.14	.18				
50	23	.20	.26	*fl oz*	*in.*	*fl oz*	*fl oz*
100	29	.35	.40	1 (8 fl dr)	3/4	0.005	0.006
250	40	.65	.80	2	1	.008	.010
500	50	1.1	1.3	4	1 1/4	.014	.017
1,000	63	2.0	2.5	8	1 9/16	.021	.027
2,000	80	3.5	5.0	16	1 15/16	.035	.045
				32	2 7/16	.060	.080

Table 13. *Limits of error in marked delivery time*

Delivery time (in seconds) less than and including—	Limit of error in marked delivery time
	sec
15	3
20	4
30	6
50	8
100	15
200	20

f. Special Apparatus

Capacity tolerances for the special purpose instruments described in section 2.4 are included in the appropriate parts of that section.

3. Special Rules for Manipulation

These rules indicate the essential points in the manipulation of volumetric apparatus which must be observed in order that the conditions necessary to obtain accurate measurements may be reproduced.

3.1. Test Liquid

Apparatus will ordinarily be calibrated with distilled water, and the capacity determined will therefore be the volume of water contained or delivered by an instrument at its standard temperature.

Certain special types of apparatus, such as pipets having a capacity of 0.01 ml or less, are more accurately calibrated with mercury. Instruments designed *to deliver* their contents should always be calibrated with water.

3.2. Method of Reading

a. Using Water or Other Wetting Liquid

In all apparatus where the volume is limited by a meniscus, the reading or setting is made on the lowest point of the meniscus. In order that the lowest point may be observed, it is necessary to place a shade of some dark material immediately below the meniscus, which renders the profile of the meniscus dark and clearly visible against a light background. A convenient device for this purpose is a collar-shaped section of thick black rubber tubing, cut open at one side and of such size as to clasp the tube firmly.

The position of the lowest point of the meniscus with reference to the graduation line is such that it is in the plane of the middle of the graduation line. This position of the meniscus is obtained by making the setting in the center of the ellipse formed by the graduation line on the front and the back of the tube as observed by having the eye slightly below the plane of the graduation line. This is illustrated in figure 5. The setting is accurate if, as the eye is raised and the ellipse narrows, the lowest point of the meniscus remains midway between the front and rear portions of the graduation line. By this method it is possible to observe the approach of the meniscus from either above or below the line to its proper setting.

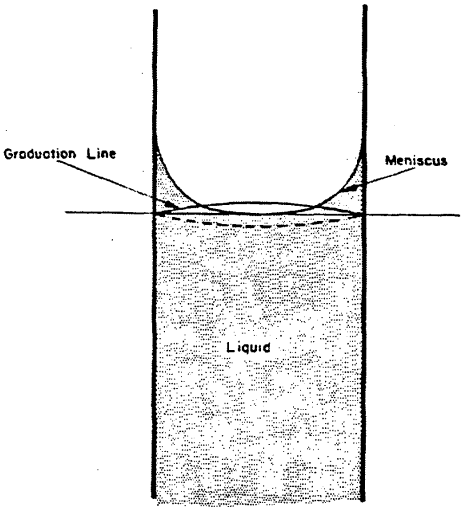


FIGURE 5. *Method of setting water meniscus.*

b. Using Mercury

In setting a mercury meniscus on a graduation line, the instrument must be tapped sharply in order that the meniscus may assume a normal shape. The *highest* point of the meniscus is set on the middle of the graduation line by employing the principles outlined for water, but observing from *above* the line.

3.3. Cleanliness of Apparatus

Apparatus must be sufficiently clean to permit uniform wetting of the surface and to minimize contamination of the liquid surface.

Of the various cleaning agents in common use, the Bureau prefers fuming sulfuric acid or a chromic-sulfuric acid mixture. For removal of oil and grease, an organic solvent is used and the cleaning completed with one of the above agents.

When drying is required before calibration, it is done with unheated compressed air which is cleaned and dried by passing through concentrated sulfuric acid and a drying compound such as calcium chloride.

3.4. Flasks and Cylinders

In filling flasks, the entire interior of the flask below the stopper will be wetted; in the case of cylinders, the liquid is allowed to flow down one side only. After filling to a point slightly below the graduation line, these instruments are allowed to drain for about 2 min. They are then placed below a buret having a long delivery tube and a bent tip, and the filling is completed by discharging water from the buret against the wall of the flask or cylinder about 1 cm above the graduation line, and rotating the receiving vessel to re-wet the wall uniformly.

Flasks and cylinders which are to be used to deliver are filled approximately to the test point, then emptied by gradually inclining them, avoiding as much as possible agitation of the contents and re-wetting of the walls. Allow half a minute for emptying. When the continuous outflow has ceased, the vessel should be nearly vertical and should be held in this position for another half-minute. The adhering drop is removed by contact with the wetted wall of the receiving vessel. The rest of the calibration is performed in the same manner as in calibrating to contain.

In calibrating a flask or cylinder to contain it is only necessary, after cleaning and drying the apparatus, to weigh it empty, fill it accurately to the graduation mark, and again weigh. Table 16 or table 17 may be used to determine the capacity at 20° C from the weight of the water contained at a known temperature.

In case flasks are to be tested in large numbers, a volumetric method may be used to advantage. The Bureau uses a series of volumetric standard pipets (illustrated in fig. 6) each having a capacity slightly less than that of the flask it is intended to test. The water delivered from the appropriate standard pipet fills the flask nearly to the graduation mark. After the 2-min drainage period, the filling is completed from an accurately calibrated buret. The capacity of the flask is then found from the known volume delivered by the standard pipet and the additional volume delivered by the buret. The standard pipets and burets are themselves calibrated by weighing the water delivered.

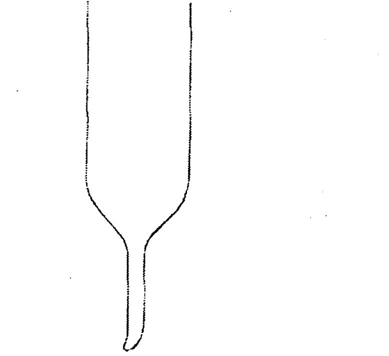


FIGURE 6. *Standard pipet for flask calibrations.*

a. Conical Graduates

The conical graduate is not classed as a precision volumetric measure, and is not recommended for use in checking the accuracy of other volumetric apparatus. The Bureau does not accept these graduates for calibration except when requested by a State weights and measures official.

Conical graduates are calibrated in the same manner as cylindrical graduates, except that in calibrating "to deliver", the emptying time is reduced from 30 sec to 10 sec and the drainage time (after emptying) is also reduced to 10 sec, following customary methods of use.

3.5. Burets

Burets are calibrated in a vertical position. After cleaning, they are filled with distilled water to about 1 cm above the zero line. They are allowed to stand for about 30 min to check for leakage, not only at the tip but also around the stopcock plug. The water is then allowed to discharge with unrestricted outflow and the delivery time is noted and recorded. This flushing serves to equalize the temperatures of apparatus and water. The water temperature is taken on the next filling by inserting a thermometer in the buret or, if it is not large enough to receive a thermometer, in a plain glass tube of larger diameter mounted near the buret.

If the temperature was taken in the buret, the thermometer is then removed and enough water added to raise the level to about 1 cm above the zero line. Any water on the outside of the tip is removed with absorbent paper. The setting of the meniscus on the zero line is made by allowing the water to run out slowly. After the setting is made, any excess water on the tip is removed by touching the tip with the wetted wall of a beaker or other vessel. The very small quantity of water which remains on the extreme end, or ground portion, of the tip is not removed, as the same quantity of water will remain after delivery into the weighing flask.

Delivery of the water into the weighing flask is made with the buret tip in contact with the inside of the flask neck, the flask being inclined at an angle of about 20°. When delivery has been completed, the weighing flask is immediately removed horizontally from contact with the instrument being calibrated. The flask is then stoppered and weighed. The buret and thermometer tube (if used) are refilled preparatory to measuring the next interval.

Buret stopcocks should be completely open during delivery until the water level is only a few millimeters above the test point, when the discharge must be slowed in order to make an accurate setting.

In making the weighings it is both convenient and accurate to use the method of substitution. By this method a constant tare is kept on one pan of the balance, while on the other pan is placed the object to be weighed and with it sufficient weights to secure equilibrium. In testing volumetric apparatus, the receiving flask is first weighed empty and then again after having delivered into it the water from the interval under test. The difference between the two weights is the weight of the water delivered.

Suppose, for example, that with a certain tare on the right-hand pan and the empty flask on the left a load of 151.276 g is required in addition to the flask to restore equilibrium, and that after the water from the 10-ml interval of the buret under test is delivered into the flask, only 141.310 g are required, and that the water is at a temperature of 23.4°C. The weight of the water added is then 151.276 − 141.310 = 9.966 g. From table 16 (appendix), it is seen that to determine the capacity of a 10-ml soft glass vessel at 20°C from the weight of water delivered at 23.4°C, 0.035 must be added to the weight; that is, the capacity of the 10-ml interval of the buret under test at 20°C is 9.966 + 0.035 = 10.001 ml.

3.6. Measuring and Transfer Pipets

Measuring and transfer pipets are calibrated in much the same manner as burets, except that the tip must be in contact with the wet wall of a beaker or other vessel when the setting is made on the zero line of a measuring pipet or the capacity mark of a transfer pipet. Also, the delivery time of a transfer pipet is measured with the tip in contact with the vessel.

In calibrating transfer pipets, the water surface must be observed closely as it approaches the tip to make certain that outflow has ceased before the weighing flask is removed. On the other hand, if the flask removal is unduly delayed, afterdrainage will affect the result.

The water remaining in the tip of a pipet is not blown out unless, as in the case of certain types of special-purpose pipets, there is a wide band or two narrower bands permanently marked near the top of the instrument.

The water temperature is measured in the beaker from which the pipet is filled (by suction).

3.7. Special Apparatus

The flasks and cylinders mentioned in section 2.4 are calibrated in the manner described in section 3.4, except that the inside of the neck is wetted for a distance of only about 1 cm or less above the test point.

A specific gravity flask is calibrated at the 1, 18, and 24 ml graduations.

Capacity pipets made *to contain* the nominal volume are calibrated by weighing before and after filling.

In calibrating "blow-out" pipets, the water remaining in the tip after free outflow has ceased is blown out, without waiting for drainage, and added to the quantity delivered. The blow-out is accomplished by a single strong puff, with the pipet tip in contact with the neck of the weighing flask.

4. Tests Performed by the Bureau

4.1. Nature of Tests

Apparatus submitted for test is first examined as to its conformity with the specifications concerning quality and workmanship, design, markings, outflow time, etc.

If the apparatus meets these requirements, it is calibrated either to ascertain whether the capacity is correct within the prescribed limits of error or to determine the correction for use in precise measurements.

4.2. Precision Stamp

If the results of the preliminary examination indicate a satisfactory conformity with the specifications, and the error in capacity is within the prescribed tolerance, the official precision stamp, consisting of the letters NBS and the year date, surrounded by a circle, is etched on the instrument as shown below:

NBS
1958

4.3. Certificates and Reports of Capacity

Burets will be calibrated for at least five intervals, and if found to conform with the specified requirements will, in addition to the precision stamp, be marked with an NBS identification number as shown below:

NBS No. 1234
1958

A certificate will be issued giving the volumes delivered from the intervals tested.

Only in special cases will certificates of capacity be issued for apparatus other than burets. When the measurements depending upon the use of the volumetric glassware are required to be so precise that a statement to the effect that the apparatus is within the tolerances prescribed in this Circular does not give sufficiently exact information, certificates may be issued. The request for certificates, when required, should be made at the time the apparatus is submitted for test.

Reports of capacity may be issued under the following circumstances:

(a) For burets when the error in capacity is greater than, but not more than twice, the tolerance.

(b) When certificates are requested for other apparatus, and the error in capacity exceeds, but is not more than twice, the prescribed tolerance.

(c) For special types of volumetric apparatus not covered by the specifications in this Circular.

When a report of capacity is issued, a number is applied as shown above, but the precision stamp is omitted.

4.4. Special Tests

The Bureau will gladly cooperate with scientific investigators, manufacturers of apparatus, and others in the calibration of precision volumetric apparatus not covered by the specifications in this Circular, as far as the regular work of the Bureau will permit. Tests of instruments not included in the current fee schedules should be arranged for by correspondence before shipment of the apparatus; the application should state fully the purpose for which the apparatus is to be used, the need for the test, and the precision desired.

5. Directions for Submitting Apparatus for Test

5.1. Application for Test

The request for test should be made in writing and should include a complete list of the apparatus and a statement of the nature of the test desired. Representatives of state institutions entitled to tests free of charge must make application in writing for each test in order to avail themselves of the privilege.

Unless otherwise requested, burets will be examined and the capacity of five intervals tested. If in satisfactory conformity with the requirements, the results of the test are certified. If more than five intervals are to be tested, the request must so state.

Patrons should always examine apparatus carefully before submitting it for test to ascertain if it complies with the construction specifications. Delay and cost of transportation on apparatus not entitled to verification will thus be avoided.

The Bureau does not sell volumetric apparatus. It may be purchased from manufacturers, importers, or jobbers, and submitted to the Bureau for test.

Purchasers of apparatus to be submitted to the Bureau for test should so specify to the dealer in order to avoid unnecessary delays and misunderstandings.

5.2. Shipping Directions

The apparatus should be securely packed in cases or packages which will not be broken in transportation and which may be used in returning the tested material to the owner. Great care should be taken in packing. Clean, dry excelsior is a suitable packing material in most cases. Each instrument should also be wrapped in strong paper or other covering to prevent dust and excelsior from getting into it.

Transportation charges are payable by the party requesting the test. The charges for shipment to the Bureau must be prepaid; and, unless otherwise arranged, articles will be returned or forwarded by express "collect".

After the material is received at the Bureau, the shipper will be notified of the test number assigned. This number should be mentioned in any correspondence pertaining to the test.

5.3. Breakage

A considerable number of pieces of glassware are received broken, either on account of improper packing or rough treatment in transportation, or both. Some are broken in return shipment. A small percentage is broken in the testing laboratory.

There is no legal way in which the Bureau can make reimbursement for breakages of this kind. It is therefore a matter of necessity, and not of choice, that the Bureau makes those who send apparatus for test assume all the risks involved. Under the circumstances, all that the Bureau can do is to make every effort to reduce such breakage to the absolute minimum.

5.4. Address

Articles and communications should be addressed, "National Bureau of Standards, Washington 25, D.C."; delays incident to other forms of addresses will thus be avoided. Articles delivered personally or by messenger should be left at the receiving office of the Bureau, and should be accompanied by a written request for test.

5.5. Remittances

Payment of test fee should be made promptly upon receipt of bill. Remittances may be made by money order or check drawn to the order of the "National Bureau of Standards".

Copies of the current Test Fee Schedules may be obtained from the Bureau upon request.

6. Appendix

Tables 14 and 16 are reprints of tables 38 and 43 from NBS Circular 19, 6th edition, Standard Density and Volumetric Tables. They are included in this Circular for the convenience of users of volumetric glassware. Tables 15 and 17 have been added because of the increasing popularity of borosilicate glass apparatus.

6.1. Temperature Corrections for Volumetric Solutions

Table 14 gives the correction to various observed volumes of water, measured at the designated temperatures, to give the volume at the standard temperature, 20° C. Conversely, by subtracting the corrections from the volume desired at 20° C, the volume that must be measured at the designated temperatures in order to give the desired volume at 20° C will be obtained. It is assumed that the volumes are measured in glass apparatus having a coefficient of cubical expansion of 0.000025/° C. The table is applicable to dilute aqueous solutions having the same coefficient of expansion as water.

Table 15 gives temperature corrections for water, when measured in borosilicate glass apparatus having a coefficient of cubical expansion of 0.000010/° C.

TABLE 14. *Temperature corrections for water*

Measured in soft glass apparatus having a coefficient of cubical expansion of 0.000025/°C

Temperature of measurement	Capacity of apparatus in milliliters at 20° C						
	2,000	1,000	500	400	300	250	150
	Correction in milliliters to give volume of water at 20° C						
° C							
15	+1.54	+0.77	+0.38	+0.31	+0.23	+0.19	+0.12
16	+1.28	+.64	+.32	+.26	+.19	+.16	+.10
17	+.99	+.50	+.25	+.20	+.15	+.12	+.07
18	+.68	+.34	+.17	+.14	+.10	+.08	+.05
19	+.35	+.18	+.09	+.07	+.05	+.04	+.03
21	−.37	−.18	−.09	−.07	−.06	−.05	−.03
22	−.77	−.38	−.19	−.15	−.12	−.10	−.06
23	−1.18	−.59	−.30	−.24	−.18	−.15	−.09
24	−1.61	−.81	−.40	−.32	−.24	−.20	−.12
25	−2.07	−1.03	−.52	−.41	−.31	−.26	−.15
26	−2.54	−1.27	−.64	−.51	−.38	−.32	−.19
27	−3.03	−1.52	−.76	−.61	−.46	−.38	−.23
28	−3.55	−1.77	−.89	−.71	−.53	−.44	−.27
29	−4.08	−2.04	−1.02	−.82	−.61	−.51	−.31
30	−4.62	−2.31	−1.16	−.92	−.69	−.58	−.35

TABLE 15. *Temperature corrections for water*

Measured in borosilicate glass apparatus having a coefficient of cubical expansion of 0.000010/°C

Temperature of measurement	Capacity of apparatus in milliliters at 20° C						
	2,000	1,000	500	400	300	250	150
	Correction in milliliters to give volume of water at 20° C						
° C							
15	+1.69	+0.84	+0.42	+0.34	+0.25	+0.21	+0.13
16	+1.39	+.70	+.35	+.28	+.21	+.17	+.10
17	+1.08	+.54	+.27	+.22	+.16	+.13	+.08
18	+0.74	+.37	+.19	+.15	+.11	+.09	+.06
19	+.38	+.19	+.10	+.08	+.06	+.05	+.03
21	−.40	−.20	−.10	−.08	−.06	−.05	−.03
22	−.83	−.41	−.21	−.17	−.12	−.10	−.06
23	−1.27	−.64	−.32	−.25	−.19	−.16	−.10
24	−1.73	−.87	−.43	−.35	−.26	−.22	−.13
25	−2.22	−1.11	−.55	−.44	−.33	−.28	−.17
26	−2.72	−1.36	−.68	−.54	−.41	−.34	−.20
27	−3.24	−1.62	−.81	−.65	−.49	−.41	−.24
28	−3.79	−1.89	−.95	−.76	−.57	−.47	−.28
29	−4.34	−2.17	−1.09	−.87	−.65	−.54	−.33
30	−4.92	−2.46	−1.23	−.98	−.74	−.62	−.37

In using the above tables to correct the volume of certain standard solutions to 20° C, more accurate results will be obtained if the numerical values of the corrections are increased by the percentages given below:

Solution	Normality		
	N	*N*/2	*N*/10
HNO_3	50	25	6
H_2SO_4	45	25	5
NaOH	40	25	5
KOH	40	20	4

6.2. Tables of Corrections for Determining the True Capacities of Glass Vessels From the Weight of Water in Air

Table 16 gives, for a nominal capacity of 100 ml and observed temperatures from 15° C to 32.9° C, the amounts to be added to the apparent weight in grams (in air against brass weights) of the water contained in or delivered by a glass vessel to give the capacity in milliliters at 20° C. It is calculated on the following data assumed as approximating ordinary conditions: Observed barometric pressure—760 mm; relative humidity—50 percent; coefficient of expansion of glass—0.000025/° C.

Example of use of table. Determination of capacity of glass volumetric flask marked "To contain 100 ml at 20° C".

Apparent weight of water at the observed temperature, 24.5° C	99.615 g
From table 16, correction	+0.372
Actual capacity at 20° C	99.987 ml

For capacities other than 100 ml, the corrections given must be multiplied by the appropriate factor. For example, the correction for 500 ml would be five times the correction for 100 ml.

For borosilicate glass having a coefficient of cubical expansion of approximately 0.000010/° C, Table 17 should be used.

6.3. Change in Capacity Caused by Change of Temperature

Having determined the capacity at 20° C, if it is desired to know what the capacity of the same vessel will be at another temperature, the following formula may be used:

$$V_t = V_{20}[1 + \alpha(t-20)],$$

where

V_t = capacity at t° C,
V_{20} = capacity at 20° C,
α = coefficient of cubical expansion of material of which the instrument is made. (For soft glass 0.000025/° C)

This formula is applicable to instruments or measures made of any material of which the coefficient of cubical expansion is known. A few materials commonly used in volumetric apparatus and their approximate coefficients are:

	Coefficient of cubical expansion[4] *per deg C*
Soft glass	0.000025
Pyrex or KG-33	.000010
Copper	.000050
Brass	.000054
Steel	.000035
Stainless steel	.000031

TABLE 16. *Table of corrections for determining the true capacities of glass vessels from the weight of water in air*

(Soft glass, coefficient of cubical expansion 0.000025/° C)

Indicated capacity 100 ml

Temperature	Tenths of degrees									
	0	1	2	3	4	5	6	7	8	9
° C										
15	0.207	0.208	0.210	0.211	0.212	0.213	0.215	0.216	0.217	0.219
16	.220	.221	.223	.224	.225	.227	.228	.230	.231	.232
17	.234	.235	.237	.238	.240	.241	.243	.244	.246	.247
18	.249	.250	.252	.253	.255	.257	.258	.260	.261	.263
19	.265	.266	.268	.270	.272	.273	.275	.277	.278	.280
20	.282	.284	.285	.287	.289	.291	.293	.294	.296	.298
21	.300	.302	.304	.306	.308	.310	.312	.314	.315	.317
22	.319	.321	.323	.325	.327	.329	.331	.333	.336	.338
23	.340	.342	.344	.346	.348	.350	.352	.354	.357	.359
24	.361	.363	.365	.368	.370	.372	.374	.376	.379	.381
25	.383	.386	.388	.390	.392	.395	.397	.399	.402	.404
26	.406	.409	.411	.414	.416	.418	.421	.423	.426	.428
27	.431	.433	.436	.438	.440	.443	.446	.448	.451	.453
28	.456	.458	.461	.463	.466	.469	.471	.474	.476	.479
29	.482	.484	.487	.490	.492	.495	.498	.501	.503	.506
30	.509	.511	.514	.517	.520	.522	.525	.528	.531	.534
31	.536	.539	.542	.545	.548	.551	.554	.556	.559	.562
32	.565	.568	.571	.574	.577	.580	.583	.586	.589	.592

---

[4] Coefficients shown are averages, but are sufficiently accurate for volumetric determinations.

Table 17. *Table of corrections for determining the true capacities of glass vessels from the weight of water in air*

(Borosilicate glass, coefficient of cubical expanson 0.000010/° C)

Indicated capacity 100 ml

Temperature	Tenths of degrees									
	0	1	2	3	4	5	6	7	8	9
° C										
15	0.200	0.201	0.202	0.204	0.205	0.207	0.208	0.210	0.211	0.212
16	.214	.215	.217	.218	.220	.222	.223	.225	.226	.228
17	.229	.231	.232	.234	.236	.237	.239	.241	.242	.244
18	.246	.247	.249	.251	.253	.254	.256	.258	.260	.261
19	.263	.265	.267	.269	.271	.272	.274	.276	.278	.280
20	.282	.284	.286	.288	.290	.292	.294	.296	.298	.300
21	.302	.304	.306	.308	.310	.312	.314	.316	.318	.320
22	.322	.324	.327	.329	.331	.333	.335	.338	.340	.342
23	.344	.346	.349	.351	.353	.355	.358	.360	.362	.365
24	.367	.369	.372	.374	.376	.379	.381	.383	.386	.388
25	.391	.393	.396	.398	.400	.403	.405	.408	.410	.413
26	.415	.418	.420	.423	.426	.428	.431	.433	.436	.438
27	.441	.444	.446	.449	.452	.454	.457	.460	.462	.465
28	.468	.470	.473	.476	.479	.481	.484	.487	.490	.492
29	.495	.498	.501	.504	.506	.509	.512	.515	.518	.521
30	.524	.526	.529	.532	.535	.538	.541	.544	.547	.550
31	.553	.556	.559	.562	.565	.568	.571	.574	.577	.580
32	.583	.586	.589	.592	.595	.598	.602	.605	.608	.611

Washington, September 24, 1958.

U. S. GOVERNMENT PRINTING OFFICE: 1959

APPENDIX VI

NBSIR 74–461

THE CALIBRATION OF SMALL VOLUMETRIC LABORATORY GLASSWARE

Josephine Lembeck

Institute for Basic Standards
National Bureau of Standards
Washington, D. C. 20234

December 1974

Final

Prepared for
Optical Physics Division
Institute for Basic Standards
Washington, D. C. 20234

U. S. DEPARTMENT OF COMMERCE, Frederick B. Dent, Secretary

NATIONAL BUREAU OF STANDARDS, Richard W. Roberts, Director

The Calibration of Small Volumetric Laboratory Glassware

by

Josephine Lembeck

1. INTRODUCTION

This article describes an acceptable procedure for calibrating burets, pipets and volumetric flasks of the types shown in figure 1. The physical characteristics of these items are indicated in Interim Federal Specifications NNN-B-00789a (GSA-FSS), NNN-P-00395a (GSA-FSS), NNN-F-00289a (GSA-FSS), and NBS Handbook 105-2.

The general procedure is based upon a determination of the volume of water either contained in or delivered by the vessel. This volume of water is based upon knowledge of its measured mass and its tabulated density. Inasmuch as these calibration volumes, especially the delivery volume, may be altered by the manner in which the vessels are used, it is imperative that the user of the vessel employ the same techniques as those employed during calibration. The techniques of importance here are:

1. reading and adjusting meniscus
2. filling the vessel
3. draining the vessel.

These three topics will be covered in detail.

2. CLEANING PROCEDURES

It is important that glassware be thoroughly cleaned before being tested or used. Apparatus must be sufficiently clean to permit uniform wetting of the surface. When clean, the walls will be uniformly wetted and the water will adhere to the glass surface in a continuous film. Imperfect wetting causes irregularities in capacity by distorting the meniscus and allowing nonuniform wetting of the interior surfaces to occur.

The liquids usually used for cleaning glassware are sodium dichromate sulphuric acid cleaning solution (commercially available from laboratory supply houses), nitric acid, fuming sulphuric acid, alcohol and water. The choice of cleaning agent to be used depends on the nature of the contaminant. After cleaning with the cleaning solution and thoroughly rinsing with tap water, the vessel should be rinsed with distilled water. If the vessel is marked "TO CONTAIN", it should additionally be rinsed with ethyl alcohol and dried with clean air at room temperature. NBS uses bottled breathing air and a drying rack shown in figure 2. It is not necessary to dry any vessel marked "TO DELIVER". When cleaning small articles such as pipets, it is usually easier to fill them with cleaning solution by suction, vacuum, if available, or a small rubber bulb, but never by mouth. The solution should be sucked through the pipet several times until the entire inside surface is evenly coated. Rinse thoroughly with tap water and then with distilled water.

For cleaning flasks, pour in enough cleaning solution while rotating so that a film of solution will cover the entire interior surface. A break in the film indicates a contaminated area. For filling a buret with cleaning solution, it should be held in a vertical position and filled by pouring into the top. Open the stopcock to drain. Regardless of the type of vessel, always rinse thoroughly, first with tap water and then with distilled water.

3. FILLING AND DRAINING THE VESSEL

Pipets, burets and flasks have at least one index mark engraved on their glass bodies which serves to define their reference volume. Calibration consists of determining the volume of water required to fill the vessel to the index mark (containment volume), or the volume of water which can be drained from the vessel under specified conditions after filling (delivery volume).

The difference between containment volume and delivery volume is significant. Since these volumes are determined in weighing experiments, it is essential to avoid splashing droplets of water on to supposedly dry surfaces and it is equally important to assure that a surface is uniformly wetted when required by the procedure.

In all cases, it is necessary to fill the reference volume of the test vessel with water. This involves adjusting the height of the liquid surface to coincidence with the horizontal plane through the defining index mark.

This surface, or meniscus, is not flat; it is concave-- that is, its lowest point is in the center of the surface as shown in figure 3. There are several criteria for deciding when coincidence is achieved. Inasmuch as differing criteria result in different surface heights, it is important that the same criteria be employed during use of the vessel as was employed during calibration. Experience has shown that the following generally used technique is easy to apply and adequately reproducible. In this procedure, the lowest point in the meniscus is brought to the same horizontal level as the horizontal plane through the engraved index line on the glass vessel.

In order to observe the lowest point, it is necessary to place a shade of some dark material immediately below the meniscus. This renders its profile dark and clearly visible against a light background. A method or device for this purpose is a collar-shaped section of thick black rubber tubing, cut open at one side and of such size as to clasp the tube firmly, as shown in figures 4 and 4A. The position of the lowest point of the meniscus with reference to the graduation line is such that it is in the plane of the middle of the graduation line. This position of the meniscus is obtained by making the setting in the center of the ellipse formed by the graduation line on the front and the back of the tube, as observed by having the eye slightly below the plane of the graduation line. The setting, the procedures for which will be discussed later, is accurate if, as the eye is raised and the ellipse narrows, the lowest point of the meniscus remains midway between the front and rear portions of the graduation line. By this method, it is possible to observe the approach of the meniscus from either above or below the line to its proper setting.

The foregoing procedure applies when the index line runs completely around the neck of the vessel. On some vessels, such as measuring flasks, figure 1, some of the lines go only one-half way around the neck. Under these conditions the operator has only one-half of an ellipse to assist him in defining the horizontal plane through the index line. Experience has shown that, although the 360° index line is most convenient for the operator, there is insignificant difference between the achievable reproducibility from this complete index, and one which extends only 180° around the neck.

On some graduated neck vessels the lines are very short indeed, extending less than one-fourth the way around the neck. Under these circumstances, there is no reference line <u>behind</u> the meniscus to help the operator in estimating the vertical position of the horizontal index plane. When faced with this problem, it is necessary for the operator to place his eye in his <u>best estimate</u> of the horizontal index plane while observing the meniscus. With experience, an operator should be able to judge the position of this plane to within one or two centimeters. The operator's eye will be (perhaps) twenty-five centimeters in front of the short index line while the center of the meniscus will be only one centimeter behind this line. (for a two centimeter neck on the vessel). The geometry of this situation dictates that the vertical position error of the meniscus will be only one twentyfifth of the operator's error in estimating the position of the horizontal index plane. Although an optical system (e.g. a bubble level cathetometer) could reduce this error to insignificance, there is little point to such a calibration procedure unless a similar system were employed by the user of the vessel.

Having filled the reference volume of the test vessel using the meniscus observation technique procedure described above, the problem is to determine the volume of water contained, or the volume which can be drained out under specified conditions. This volume is calculated from the density of the water from table 2 and its measured mass.

In the case of a flask "to contain," this mass is the difference between the mass of the empty flask and its mass after filling as described above. For the flask "to deliver" it is the difference between the mass of the filled flask and the mass after the specified drainage period, 30 seconds, has occurred <u>but before the walls have had a chance to become dry</u>.

It is not necessary to weigh burets and pipets because they are "delivery" instruments. Under these circumstances their delivery volume is drained into a weighing flask. Weighing this flask, before and after loading, provides the mass of water delivered by the buret or pipet.

The detailed procedures in use at NBS for filling and draining the various test vessels are given in the following paragraphs.

A. BURETS

Clamp the buret vertically on a support stand; also clamp a plain glass tube, large enough to hold a thermometer, near the buret if the buret is of such a size that is not large enough to insert a thermometer in the top for observing the temperature of the water. A typical setup is shown in figure 5. Fill the buret from the top to the zero mark with distilled water from a reservoir or storage bottle. Drain and record the delivery time. Delivery time is determined by the unrestricted outflow of the water from the zero mark to the lowest graduation mark with the stopcock fully open. Refill the buret to approximately 1 cm above the zero mark and fill the plain glass tube that holds the thermometer; record the temperature. Set a meniscus on the zero mark using the buret stopcock to lower the liquid level and touch the tip with the wetted wall of a beaker to remove any excess water. A weighing flask which has been lightly stoppered and weighed empty is placed with the inside of the neck in contact with the tip of the buret (the flask will be at a slight angle). The stopcock must be fully open until the water is only a few millimeters above the line being tested and then slowed so as to make an accurate setting. When the setting has been completed, move the flask horizontally, breaking contact with the buret. The flask is then stoppered and weighed a second time, after which the thermometer tube is refilled and the next interval tested in the same manner as the first one - from the zero mark to the next interval needed.

B. PIPETS

Fill pipet with distilled water by suction to the index mark and measure delivery time with the tip in contact with the glass surface or the internal side of a beaker as shown in figure 6. Refill by suction slightly above index line. Record the temperature of the distilled water in the beaker from which the pipet is filled. Any water on the outside of the tip is removed with filter paper after the filling is completed. The meniscus is then slowly lowered to the index using either the stopcock or hose clamp for "fine control." The tip must be in contact with the wet wall of a beaker while the setting is made on the index line. Any water remaining on the tip at this time is not removed. The pipet is held in a vertical position while water is delivered into a previously weighed weighing flask with the tip in contact with the inside wall of the neck of the flask as indicated in the figure 6A. Contact is broken immediately after outflow ceases. The flask is now stoppered and weighed with its contained load.

C. FLASKS (TO CONTAIN)

After cleaning and drying, weigh the empty flask including the stopper. Place an appropriate sized funnel in the flask to discharge the water below the stopper. Fill from beaker or supply line to a point slightly below the graduation line, maneuvering the funnel so as to wet the entire neck below the stopper. Let stand for about two minutes to allow the walls to drain. Place the flask under the buret and complete filling and setting the meniscus and place stopper in neck to lessen evaporation and weigh. A pipet or dropper with a finely drawn tip may be used instead of the buret. Determine the temperature by placing a thermometer in the filling beaker or in one which has been filled from the supply line. The temperature may be taken after final weighing by placing a thermometer directly in the flask, provided the flask is of sufficient size to accommodate it.

D. FLASKS (TO DELIVER)

Flasks calibrated to deliver are not dried prior to the test. Fill slightly below the index line and empty in 30 seconds by gradually inclining the flask so as to avoid splashing of the walls as much as possible. When the main drainage stream has ceased, the flask will be nearly vertical. Hold in this position another 30 seconds and touch off the drop of water adhering to the top of the flask. Place a stopper, or cap, on the flask to reduce evaporation and weigh immediately. Take water temperature and fill the flask, and make the meniscus setting on the index line. Place a cap, or stopper, on the filled flask and weigh.

4. WEIGHING PROCEDURE AND CALCULATIONS

Most laboratories employ single-pan direct-reading balances for their weighing work. Although the manufacturers' instructions for use differ in minor detail from brand-to-brand, the major operating features are essentially the same. Most instruments are equipped with a screen upon which an image is projected of a small reticle attached to the beam. Under ordinary circumstances, a slight "zero" adjustment is made to the screen reading with the balance pan empty. When the pan is loaded by the unknown object, the operator manipulates dials which mechanically add or remove built-in weights from the internal balance structure. On some balances, the screen readings will be "off-scale," either high or low, depending upon whether the operator should increase or decrease the dial-operated load. When the screen is "on-scale" the balance is ready to read. Some balances have colored lights to indicate whether an increase or decrease of built-in load is called for. The more sensitive direct-reading balances are constrained from swinging freely until the proper dial-operated load is in place. This condition is called "partial arrestment." On the other hand, the relatively insensitive top-loading balances (used when the physical size of the weighing flask is large) are never arrested except when the balance is being moved.

When the manual adjustment of the dial-operated load is complete, the numerical balance indication should be observed in accordance with the manufacturer's instructions. The coarse decades are usually observed on the weight manipulation dials, and the last two (or three) decades are projected on the screen. There are two such indications, I_E referring to the empty vessel, and I_L, the loaded vessel. The indication referring to the water is obtained by subtraction:

$$I_w = I_L - I_E$$

If I_E and I_L are observed under the same conditions the zero adjustment, called for in the operating instructions, may be eliminated.

These numerical indications are normally <u>not</u> the mass of the unknown. They are a measure on an arbitrary scale called

Apparent Mass (see appendix) of the load of built-in weights which exert the same force on the balance as the unknown load under the ambient conditions existing at the instant of the weighing. We can write an equation indicating the equality of the forces exerted on the balance by the water and the built-in weights associated with I_w. After eliminating "g" from both sides, we have

$$M_w\left[1 - \frac{\rho_A}{\rho_w}\right] = M_B\left[1 - \frac{\rho_A}{\rho_B}\right] \tag{1}$$

where M_B and ρ_B are the mass and density of the built-in weights, M_w the mass of water, and ρ_A the air density at the instant of measurement. If we rearrange terms and divide both sides by the density of water, we obtain an expression for the sought after volume of water, V.

$$V = M_B\left[\frac{1}{\rho_w - \rho_A}\right]\left[1 - \frac{\rho_A}{\rho_B}\right] \tag{2}$$

Although the volume, V, in equation (2) is the volume of water in the weighing flask at the instant of the weighing, <u>it is also</u> the calibrated volume of the vessel under test at temperature, T. To obtain this volume at 20 °C (the usual reference temperature) requires multiplication by the thermal expansion factor, K, which involves the test temperature, T, and the temperature coefficient of cubical expansion, α, of the material from which the vessel is made, thus,

$$K = 1 - \alpha(T-20)$$

and

$$V_{20} = M_B\left[\frac{1}{\rho_w - \rho_A}\right]\left[1 - \frac{\rho_A}{\rho_B}\right]\left[K\right] \tag{3}$$

As explained in the appendix, the mass of the balance weights, M_B, which exert the same force as the water is given by the product of the balance indication for the water, I_w, and the apparent mass conversion factor, Q, where

$$I_w = I_L - I_E$$

$$Q = \frac{\rho_B D_{20} - .0012\rho_B}{\rho_B D_{20} - .0012 D_{20}}$$

and

$$V_{20} = \left[I_L - I_E\right]\left[Q\right]\left[\frac{1}{\rho_w - \rho_A}\right]\left[1 - \frac{\rho_A}{\rho_B}\right]\left[K\right] \tag{4}$$

Equation (4) shows the numerical value of the calibration, V_{20}, to be the product of five easily evaluated terms.

TERM 1 $\left[I_L - I_E\right]$	is the difference obtained by subtracting the balance indication in grams associated with the empty weighing flask from that associated with the loaded flask.
TERM 2 $[Q]$	is the apparent mass conversion factor. An acceptable value may be found in table 3 based upon the density of the balance weights, ρ_B, and the apparent mass scale to which they are adjusted, D_{20}. Both ρ_B and D_{20} may be obtained from the manufacturer of the balance if the information is not on the name plate. This value need be calculated only once for each balance.
TERM 3 $\left[\frac{1}{\rho_W - \rho_A}\right]$	There are two density terms which require knowledge of air density, ρ_A, water density, ρ_w, and the density of the balance weights, ρ_B. Air density may be adequately estimated by table 1. Water density is obtained from table 2. The density of the balance weights, ρ_B, is obtained from the balance maker. His quoted value will be the density at 20°C. Inasmuch as the test temperature, T, will be near 20° and the coefficient of expansion is small the quoted value is adequate for this work.
TERM 4 $\left[1 - \frac{\rho_A}{\rho_B}\right]$	
TERM 5 $[K]$	The thermal expansion factor is obtainable from table 4 based upon water temperature, T_w, assumed equal to test temperature, T, and the coefficient of expansion of the vessel, α.

A sample calculation based on typical data is shown on the flow diagram, figure 7.

Examination of these numerical values shows that the value of V_{20}, equation 4, is primarily dependent upon the term showing the two balance indications, $I_L - I_E$. The other four terms, all near unity, are corrections to the balance term for the various factors mentioned above. For calibration work which is satisfied by an uncertainty of a percent or so, these four terms may be ignored.

Use of this complete format is a tedious task which requires the use of four tables and the performance of a five-multiplier product. This is because the format is designed to accommodate a very wide range of input data. However, this format offers a number of advantages:

a. It satisfies the requirements for the calibration of fused silica vessels of enormous thermal stability as well as plastic vessels of extreme thermal sensitivity.

b. It accepts data from the rather old direct-reading balances which were equipped with brass weights as well as the modern instruments with steel weights.

c. It is adaptable to balances having built-in weights adjusted to the old apparent mass vs 8.3909 scale and the new apparent mass vs 8.0 scale.

There are few, if any, calibration laboratories which work on such a wide variety of vessels with so many types of instruments. The data reduction may be grossly simplified to one table and the performance of one multiplication by restricting its applicability to the work most frequently performed.

For example, the vast majority of vessels which are submitted to NBS for calibration are made of borosilicate glass so we may eliminate α as a variable and assign to it a constant value of 0.000 010/°C so that K becomes a single-valued function of temperature,

$$K = 1 - 0.000\,010\ (T-20).$$

Additionally, all balances in the glassware calibration laboratory have built-in weights whose densities at 20 °C are very nearly 7.78 g/cm^3, so we may eliminate ρ_B as a variable, assigning to it the constant value

$$\rho_B = 7.78\ \text{g/cm}^3.$$

The built-in weights of these balances have all been adjusted to apparent mass vs 8.3909, so that we may eliminate Q as a variable, assigning to it a constant value of

$$Q = 1.000\,011\,2.$$

Without significant error we may assign values for the density of air and of water of

$$\rho_A = \frac{0.464554B - 40(.00252T - 0.020582)}{1000(T + 273.16)}$$

where B = barometric pressure in millimeters of mercury and the value of relative humidity is assumed to be 40%. [1]*

---

* Numbers in brackets refer to similarly numbered references at the end of this paper.

$$\rho_w = \left[1 - \frac{(T - 3.9863)^2}{508929.2} \times \frac{T + 288.9414}{T + 68.12963}\right](0.999973)$$

which is the Tilton-Taylor formula [2] expressed in g/cm^3.

The four correction terms given in equation 4 are now reduced to experimental constants or quantities which may be expressed in terms of measured barometric pressure, B, and/or measured temperature, T. If Z is the product of these four terms

$$Z = \left[1.000\ 011\ 2\right]\left[\frac{1}{\rho_w - \rho_A}\right]\left[1 - \frac{\rho_A}{7.78}\right]\left[1 - 0.000\ 010(T-20)\right] \qquad (5)$$

The right member of equation 5 may be put into the form of a numerical table entered with measured values of temperature and pressure from which a value of Z may be selected, then

$$V_{20} = \left[I_L - I_E\right]Z \qquad (6)$$

The table of values of Z must necessarily span the temperature range found in the calibration laboratory. Table 5 is an abbreviated form of the table used in NBS glassware calibration. For the restricted conditions mentioned above (i.e. borosilicate glass vessels calibrated by balances with weights of 7.78 g/cm^3 density adjusted to apparent mass vs. 8.3909) table 5 and equation 6 may be used with no loss of accuracy.

5. THE USE OF EQUAL-ARM BALANCES

Laboratories which employ equal-arm balances in the calibration of glassware may find adequate procedures in NBS Circular 602 "Testing of Glass Volumetric Apparatus" by J.C. Hughes.*

---

* This publication is out-of-print but is available for loan at most Government Depository Libraries throughout the country.

Appendix 1. Apparent Mass

Apparent mass scales (there are two in current use) are attempts to simplify the calibration and use of weights. They are discussed in detail by Pontius [3]. Their background is of no interest here but the concept should be understood because the built-in weights of direct-reading balances are adjusted to nominal values of apparent mass. If water volumes are to be calculated from knowledge of density and the results of weighing tests, the weighing results must be in mass, not apparent mass.

The observed numerical indication, I, obtained from a measurement on a direct-reading balance is the apparent mass of the effective dial-operated load pertaining to that measurement. The effective load is the sum of the active built-in weights plus a small additional amount observed on the screen. It is necessary to convert observed values of I from units of apparent mass into units of mass. This conversion follows directly from the definition of apparent mass.

> DEFINITION: When, under specified ambient conditions, an unknown object exerts the same force on a balance as mass, M, of a specified hypothetical reference material, the object is said to have an apparent mass versus the reference material equal to M*, where M* is numerically equal to M. The specified ambient conditions are (1) temperature = 20°C, (2) air density = 0.0012 g/cm^3. The hypothetical reference material is completely specified by its density at 20°C. For the older apparent mass scale, this specified density at 20°C, D_{20}, is 8.3909 g/cm^3, and for the more recent scale, 8.0.

By convention M* is used when the apparent mass scale is referred to density 8.3909 material, and M** when referred to 8.0 density material.

In the conversion procedure, the "unknown object" referred to in the definition is the effective dial-operated load whose mass, M, is to be calculated. The defining equality between the forces exerted by the effective dial-operated load and by the mass, I, of the hypothetical reference material is

$$M\left[1 - \frac{.0012}{\rho_B}\right] = I\left[1 - \frac{.0012}{D_{20}}\right]$$

where ρ_B is the density of the built-in weights. The 0.0012 is the specified air density which must exist during the defining equality. The mass of the effective dial-operated load is then

$$M = I\left[\frac{\rho_B D_{20} - .0012\rho_B}{\rho_B D_{20} - .0012D_{20}}\right]$$

Values of ρ_B and D_{20}, if not shown in the balance instruction book, are available from the manufacturer.

If $$Q = \left[\frac{\rho_B D_{20} - .0012\rho_B}{\rho_B D_w - .0012D_{20}}\right]$$

then $$M = IQ$$

Values of Q for various values of ρ_B and D_{20} are given in table 3.

Appendix 2

The nominal values of the reference volumes of vessels are etched on the glass walls, and may be in units of milliliters or cubic centimeters. While, strictly speaking, milliliters are no longer an acceptable unit, for the purposes of this report, they will be considered as identical to cubic centimeters. The basic SI unit of volume is the cubic meter, M^3. Due to its large size, it is rarely employed in glassware calibration work.

Experience has shown that a competent operator should repeat volumetric calibrations within the limits indicated below.

VESSEL	NOMINAL SIZE	REPRODUCIBILITY*
Transfer Pipet	1 cm^3	0.002 cm^3
	2	.002
	5	.002
	10	.003
	15	.005
	25	.005
	50	.007
	100	.010
Flasks	10 cm^3	0.005 cm^3
	25	.005
	50	.007
	100	.011
	200	.014
	250	.017
	500	.021
	1000	.042
Burets	10 cm^3	0.003 cm^3
	25	.005
	50	.007
	100	.012

* The term "reproducibility" means the maximum deviation expected between two independent determinations of the volume. To determine the reproducibility of one's process one should recalibrate one of his own vessels periodically (e.g. once a month) to obtain a sequence of values V_1, V_2, V_3,.....V_n. From these values one can compute an average $\overline{V}$ and a standard deviation s.d. = $\sqrt{\Sigma(V-\overline{V})^2/(n-1)}$ and use the quantity $3(\sqrt{2})$(s.d.) as the reproducibility of his process. It is essential that the recalibration be done far enough apart in time to be truly independent and representative of the process and the random errors which affect it.

The volumetric error associated with a faulty meniscus observation is a function of the magnitude of the observation error and the cross section area of the vessel's neck.

Some typical values are given below.

Error in Meniscus Position	Typical Neck Diameters			
	0.5 cm	1 cm	2 cm	3 cm
0.005 cm	0.001 cm^3	.004 cm^3	.016 cm^3	.035 cm^3
0.01 "	.002 "	.008 "	.031 "	.071 "
0.05 "	.010 "	.039 "	.157 "	.353 "
0.10 "	.020 "	.078 "	.314 "	.707 "
0.20 "	.039 "	.157 "	.628 "	1.414 "

REFERENCES

[1] Bowman, H.A. and Schoonover, R.M., Procedure for High Precision Density Determinations by Hydrostatic Weighing, J. Res. Nat. Bur. Stand. (U.S.), 71C (Eng. and Instr.), No. 3, pp. 179-198, (July-Aug. 1967).

[2] Tilton, L.W. and Taylor, J.K., Accurate Representation of the Refractivity and Density of Distilled Water as a Function of Temperature, J. Res. Nat. Bur. Stand. (U.S.), 18, RP971, pp. 205-213 (Feb. 1937).

[3] Pontius, P.E., Mass and Mass Values, Nat. Bur. Stand. (U.S.) Monogr. 133, 33 pages (1974).

TABLE 1

Air Density

TABLE 1A.

Listed below in table 1A are the approximate average Winter (January) and Summer (June-July) air densities for a group of selected cities throughout the continental United States. These densities are for an assumed temperature of 23°C and are based on information about the barometric pressure and relative humidity supplied by the Weather Bureau.

It should be remembered that these are average air densities and that the actual air density at a given time and place may differ from that given by as much as 3 percent in either direction, which may result in errors in V_{20} of 0.05%.

This data may be used as a guide in estimating values of ρ_A required for the calculation of V_{20}, equation 4.

NOTE CAREFULLY

ρ_A in equation 4 must be expressed in g/cm^3 and the values tabulated below are in mg/cm^3. Therefore, tabulated values must be multiplied by 0.001 (1.18 mg/cm^3 becomes 0.00118 g/cm^3).

This table is taken from Appendix 1 in NBS Monograph 133, Mass and Mass Values, by Paul E. Pontius.

TABLE 1B.

This table provides values of air density calculated from temperature, barometric pressure and assumed relative humidity of 40%. Errors in V_{20} which result from use of this table are insignificant.

TABLE 1A

Place		Air density	
State	City	Winter	Summer
		(mg/cm^3)	(mg/cm^3)
Alabama	Birmingham	1.16	1.16
	Montgomery	1.18	1.18
Arizona	Flagstaff	0.92	0.93
	Phoenix	1.15	1.14
	Tucson	1.08	1.08
	Yuma	1.19	1.18
Arkansas	Little Rock	1.18	1.18
California	Los Angeles	1.17	1.16
	Oakland	1.19	1.16
	San Diego	1.18	1.18
	San Francisco	1.18	1.18
Colorado	Denver	0.98	0.98
	Grand Junction	1.00	1.00
	Pueblo	1.00	1.00
Connecticut	Hartford	1.18	1.18
District of Columbia.	Washington	1.19	1.18
Florida	Jacksonville	1.19	1.18
	Key West	1.19	1.18
	Miami	1.19	1.18
	Tampa	1.19	1.18
Georgia	Atlanta	1.15	1.14
	Augusta (Aiken, S.C.)	1.18	1.18
	Savannah	1.19	1.18
Idaho	Boise	1.07	1.07
Illinois	Cairo	1.18	1.17
	Chicago	1.16	1.16
	Moline	1.17	1.17
	Springfield	1.16	1.16
Indiana	Fort Wayne	1.15	1.15
	Indianapolis	1.15	1.15
Iowa	Burlington	1.16	1.15
	Des Moines	1.15	1.15
	Dubuque	1.16	1.15
	Sioux City	1.15	1.13
Kansas	Concordia	1.13	1.12
	Dodge City	1.08	1.08
	Topeka	1.15	1.15
	Wichita	1.14	1.13
Kentucky	Louisville	1.17	1.17
Louisiana	New Orleans	1.19	1.18
	Shreveport	1.18	1.18
Maine	Eastport	1.18	1.18
Massachusetts	Boston	1.18	1.18
Michigan	Alpena	1.16	1.16
	Detroit	1.16	1.15
	Grand Rapids	1.15	1.15
	Marquette	1.15	1.15
	Sault Ste. Marie	1.16	1.16
Minnesota	Duluth	1.14	1.14
	Minneapolis	1.15	1.15
	St. Paul (Airport)	1.15	1.15
Mississippi	Vicksburg	1.18	1.18
Missouri	Kansas City	1.15	1.15
	St. Louis	1.17	1.16
	Springfield	1.14	1.13
Montana	Havre	1.08	1.08
	Helena	1.02	1.02
	Kalispell	1.03	1.03
Nebraska	Lincoln	1.14	1.14
	North Platte	1.07	1.07
Nevada	Ely	0.95	0.96
	Las Vegas	1.11	1.11
New Hampshire	Concord	1.17	1.17
New Jersey	Newark	1.19	1.19
New Mexico	Albuquerque	1.00	1.00
New York	Albany	1.18	1.18
	Buffalo	1.16	1.15
North Carolina	Hatteras	1.18	1.17
	Raleigh	1.18	1.17
North Dakota	Bismarck	1.11	1.11
	Devils Lake	1.12	1.12
	Fargo	1.15	1.15
	Williston	1.11	1.11
Ohio	Cincinnati	1.16	1.16
	Dayton	1.15	1.15
	Cleveland	1.15	1.15
Oklahoma	Oklahoma City	1.14	1.14
Oregon	Baker	1.04	1.04
	Medford	1.13	1.14
	Portland	1.18	1.19
	Roseburg	1.17	1.17
Pennsylvania	Philadelphia	1.18	1.18
Rhode Island	Providence	1.18	1.18
South Carolina	Charleston	1.19	1.18
	Columbia	1.18	1.17
South Dakota	Huron	1.14	1.13
	Rapid City	1.05	1.06
Tennessee	Knoxville (Oak Ridge).	1.16	1.15
	Memphis	1.18	1.17
	Nashville	1.17	1.17
Texas	Abilene	1.12	1.11
	Amarillo	1.04	1.04
	Austin	1.17	1.16
	Brownsville	1.19	1.18
	Fort Worth	1.16	1.16
	El Paso	1.04	1.04
	Houston	1.18	1.18
	San Antonio	1.17	1.17
Utah	Salt Lake City	1.02	1.02
Vermont	Burlington	1.17	1.17
Virginia	Norfolk	1.19	1.18
	Richmond	1.18	1.18
Washington	Seattle	1.18	1.17
	Spokane	1.19	1.18
	Walla Walla	1.15	1.13
West Virginia	Elkins	1.11	1.11
	Parkersburg	1.16	1.16
Wisconsin	Green Bay	1.16	1.16
	Madison	1.15	1.15
	Milwaukee	1.16	1.15
Wyoming	Casper	0.97	0.97
	Cheyenne	0.94	0.96
	Sheridan	1.03	1.04

TABLE 1B

BAROMETRIC PRESSURE (MM OF HG)	16 °C	18 °C	20 °C	22 °C	24 °C	26 °C	28 °C
600	0.00096	0.00095	0.00095	0.00094	0.00093	0.00093	0.00092
605	0.00097	0.00096	0.00095	0.00095	0.00094	0.00093	0.00093
610	0.00098	0.00097	0.00096	0.00096	0.00095	0.00094	0.00093
615	0.00098	0.00098	0.00097	0.00096	0.00096	0.00095	0.00094
620	0.00099	0.00099	0.00098	0.00097	0.00096	0.00096	0.00095
625	0.00100	0.00099	0.00099	0.00098	0.00097	0.00096	0.00096
630	0.00101	0.00100	0.00099	0.00099	0.00098	0.00097	0.00097
635	0.00102	0.00101	0.00100	0.00099	0.00099	0.00098	0.00097
640	0.00102	0.00102	0.00101	0.00100	0.00100	0.00099	0.00098
645	0.00103	0.00103	0.00102	0.00101	0.00100	0.00100	0.00099
650	0.00104	0.00103	0.00103	0.00102	0.00101	0.00100	0.00100
655	0.00105	0.00104	0.00103	0.00103	0.00102	0.00101	0.00100
660	0.00106	0.00105	0.00104	0.00103	0.00103	0.00102	0.00101
665	0.00106	0.00106	0.00105	0.00104	0.00103	0.00103	0.00102
670	0.00107	0.00107	0.00106	0.00105	0.00104	0.00103	0.00103
675	0.00108	0.00107	0.00107	0.00106	0.00105	0.00104	0.00103
680	0.00109	0.00108	0.00107	0.00107	0.00106	0.00105	0.00104
685	0.00110	0.00109	0.00108	0.00107	0.00107	0.00106	0.00105
690	0.00110	0.00110	0.00109	0.00108	0.00107	0.00107	0.00106
695	0.00111	0.00110	0.00110	0.00109	0.00108	0.00107	0.00107
700	0.00112	0.00111	0.00110	0.00110	0.00109	0.00108	0.00107
705	0.00113	0.00112	0.00111	0.00110	0.00110	0.00109	0.00108
710	0.00114	0.00113	0.00112	0.00111	0.00110	0.00110	0.00109
715	0.00115	0.00114	0.00113	0.00112	0.00111	0.00110	0.00110
720	0.00115	0.00114	0.00114	0.00113	0.00112	0.00111	0.00110
725	0.00116	0.00115	0.00114	0.00114	0.00113	0.00112	0.00111
730	0.00117	0.00116	0.00115	0.00114	0.00114	0.00113	0.00112
735	0.00118	0.00117	0.00116	0.00115	0.00114	0.00114	0.00113
740	0.00119	0.00118	0.00117	0.00116	0.00115	0.00114	0.00113
745	0.00119	0.00118	0.00118	0.00117	0.00116	0.00115	0.00114
750	0.00120	0.00119	0.00118	0.00118	0.00117	0.00116	0.00115
755	0.00121	0.00120	0.00119	0.00118	0.00118	0.00117	0.00116
760	0.00122	0.00121	0.00120	0.00119	0.00118	0.00117	0.00117
765	0.00123	0.00122	0.00121	0.00120	0.00119	0.00118	0.00117
770	0.00123	0.00122	0.00122	0.00121	0.00120	0.00119	0.00118
775	0.00124	0.00123	0.00122	0.00122	0.00121	0.00120	0.00119
780	0.00125	0.00124	0.00123	0.00122	0.00121	0.00121	0.00120
785	0.00126	0.00125	0.00124	0.00123	0.00122	0.00121	0.00120
790	0.00127	0.00126	0.00125	0.00124	0.00123	0.00122	0.00121
795	0.00127	0.00126	0.00126	0.00125	0.00124	0.00123	0.00122

T	0.0°C	0.1°C	0.2°C	0.3°C	0.4°C	0.5°C	0.6°C	0.7°C	0.8°C	0.9°C
.0	.999840	.999846	.999853	.999859	.999865	.999871	.999877	.999883	.999888	.999893
1.0	.999899	.999903	.999908	.999913	.999917	.999921	.999925	.999929	.999933	.999937
2.0	.999940	.999943	.999946	.999949	.999952	.999954	.999956	.999959	.999961	.999963
3.0	.999964	.999966	.999967	.999968	.999969	.999970	.999971	.999971	.999972	.999972
4.0	.999972	.999972	.999972	.999971	.999971	.999970	.999969	.999968	.999967	.999965
5.0	.999964	.999962	.999960	.999958	.999956	.999954	.999951	.999949	.999946	.999943
6.0	.999940	.999937	.999933	.999930	.999926	.999922	.999918	.999914	.999910	.999906
7.0	.999901	.999896	.999892	.999887	.999881	.999876	.999871	.999865	.999860	.999854
8.0	.999848	.999842	.999835	.999829	.999822	.999816	.999809	.999802	.999795	.999787
9.0	.999780	.999773	.999765	.999757	.999749	.999741	.999733	.999725	.999716	.999707
10.0	.999699	.999690	.999681	.999672	.999662	.999653	.999643	.999634	.999624	.999614
11.0	.999604	.999594	.999583	.999573	.999562	.999552	.999541	.999530	.999519	.999507
12.0	.999496	.999485	.999473	.999461	.999449	.999437	.999425	.999413	.999401	.999388
13.0	.999376	.999363	.999350	.999337	.999324	.999311	.999297	.999284	.999270	.999256
14.0	.999243	.999229	.999215	.999200	.999186	.999172	.999157	.999142	.999128	.999113
15.0	.999098	.999083	.999067	.999052	.999036	.999021	.999005	.998989	.998973	.998957
16.0	.998941	.998925	.998908	.998892	.998875	.998858	.998841	.998824	.998807	.998790
17.0	.998773	.998755	.998738	.998720	.998702	.998684	.998666	.998648	.998630	.998612
18.0	.998593	.998575	.998556	.998537	.998519	.998500	.998480	.998461	.998442	.998422
19.0	.998403	.998383	.998364	.998344	.998324	.998304	.998284	.998263	.998243	.998222
20.0	.998202	.998181	.998160	.998139	.998118	.998097	.998076	.998055	.998033	.998012
21.0	.997990	.997968	.997947	.997925	.997903	.997881	.997858	.997836	.997814	.997791
22.0	.997768	.997746	.997723	.997700	.997677	.997654	.997630	.997607	.997584	.997560
23.0	.997536	.997513	.997489	.997465	.997441	.997417	.997392	.997368	.997344	.997319
24.0	.997294	.997270	.997245	.997220	.997195	.997170	.997145	.997119	.997094	.997068
25.0	.997043	.997017	.996991	.996966	.996940	.996913	.996887	.996861	.996835	.996808
26.0	.996782	.996755	.996728	.996702	.996675	.996648	.996621	.996593	.996566	.996539
27.0	.996511	.996484	.996456	.996428	.996401	.996373	.996345	.996316	.996288	.996260
28.0	.996232	.996203	.996175	.996146	.996117	.996088	.996060	.996031	.996001	.995972
29.0	.995943	.995914	.995884	.995855	.995825	.995795	.995765	.995736	.995706	.995676
30.0	.995645	.995615	.995585	.995554	.995524	.995493	.995463	.995432	.995401	.995370
31.0	.995339	.995308	.995277	.995246	.995214	.995183	.995151	.995120	.995088	.995056
32.0	.995024	.994992	.994960	.994928	.994896	.994864	.994831	.994799	.994766	.994734
33.0	.994701	.994668	.994635	.994602	.994569	.994536	.994503	.994470	.994436	.994403
34.0	.994369	.994336	.994302	.994268	.994234	.994201	.994167	.994132	.994098	.994064
35.0	.994030	.993995	.993961	.993926	.993891	.993857	.993822	.993787	.993752	.993717
36.0	.993682	.993647	.993611	.993576	.993541	.993505	.993469	.993434	.993398	.993362
37.0	.993326	.993290	.993254	.993218	.993182	.993146	.993109	.993073	.993036	.993000
38.0	.992963	.992926	.992889	.992852	.992815	.992778	.992741	.992704	.992667	.992629
39.0	.992592	.992554	.992517	.992479	.992442	.992404	.992366	.992328	.992290	.992252

TABLE 2: Density of Air Free Water in g/cm^3 as a Function of the Celsius Temperature Scale, Based on the Work by H. Wagenbreth and W. Blanke, PTB-Mitteilungen 6-71.

ρ_B	Q when $D_{20} = 8.0000$ g/cm^3	Q when $D_{20} = 8.3909$ g/cm^3
7.70	1.0000058	1.0000128
7.72	1.0000054	1.0000124
7.74	1.0000050	1.0000120
7.76	1.0000046	1.0000116
7.78	1.0000042	1.0000112
7.80	1.0000038	1.0000108
7.82	1.0000035	1.0000104
7.84	1.0000031	1.0000101
7.86	1.0000027	1.0000097
7.88	1.0000023	1.0000093
7.90	1.0000019	1.0000089
7.92	1.0000015	1.0000085
7.94	1.0000011	1.0000081
7.96	1.0000008	1.0000077
7.98	1.0000004	1.0000074
8.00	1.0000000	1.0000070
8.02	0.9999996	1.0000066
8.04	0.9999993	1.0000062
8.06	0.9999989	1.0000059
8.08	0.9999985	1.0000055
8.10	0.9999981	1.0000051
8.12	0.9999978	1.0000048
8.14	0.9999974	1.0000044
8.16	0.9999971	1.0000040
8.18	0.9999967	1.0000037
8.20	0.9999963	1.0000033
8.22	0.9999960	1.0000030
8.24	0.9999956	1.0000026
8.26	0.9999953	1.0000023
8.28	0.9999949	1.0000019
8.30	0.9999946	1.0000016
8.32	0.9999942	1.0000012
8.34	0.9999939	1.0000009
8.36	0.9999935	1.0000005
8.38	0.9999932	1.0000002
8.40	0.9999929	0.9999998

TABLE 3: Values of the apparent mass conversion factor, Q, defined in the appendix as

$$Q = \frac{\rho_B(D_{20} - .0012)}{D_{20}(\rho_B - .0012)}$$

when ρ_B is the density of the balance weights in g/cm^3, and D_{20} is the apparent mass scale to which the weights are adjusted.

Water Temp (°C)	Fused Silica (Quartz) α=1.6	Boro-Silicate Glass α=10	Soft Glass α=25	Poly-Propylene Plastic α=240	Poly-Carbonate Plastic α=450
18.0	1.000003	1.000020	1.000050	1.000480	1.000900
18.5	1.000002	1.000015	1.000038	1.000360	1.000675
19.0	1.000002	1.000010	1.000025	1.000240	1.000450
19.5	1.000001	1.000005	1.000013	1.000120	1.000225
20.0	1.000000	1.000000	1.000000	1.000000	1.000000
20.5	0.999999	0.999995	0.999988	0.999880	0.999775
21.0	0.999998	0.999990	0.999975	0.999760	0.999550
21.5	0.999998	0.999985	0.999963	0.999640	0.999325
22.0	0.999997	0.999980	0.999950	0.999520	0.999100
22.5	0.999996	0.999975	0.999938	0.999400	0.998875
23.0	0.999995	0.999970	0.999925	0.999280	0.998650
23.5	0.999994	0.999965	0.999913	0.999160	0.998425
24.0	0.999994	0.999960	0.999900	0.999040	0.998200
24.5	0.999993	0.999955	0.999888	0.998920	0.997975
25.0	0.999992	0.999950	0.999875	0.998800	0.997750
25.5	0.999991	0.999945	0.999863	0.998680	0.997525
26.0	0.999990	0.999940	0.999850	0.998560	0.997300
26.5	0.999990	0.999935	0.999838	0.998440	0.997075
27.0	0.999989	0.999930	0.999825	0.998320	0.996850
27.5	0.999988	0.999925	0.999813	0.998200	0.996625
28.0	0.999987	0.999920	0.999800	0.998080	0.996400
28.5	0.999986	0.999915	0.999788	0.997960	0.996175
29.0	0.999986	0.999910	0.999775	0.997840	0.995950
29.5	0.999985	0.999905	0.999763	0.997720	0.995725
30.0	0.999984	0.999900	0.999750	0.997600	0.995500

TABLE 4: Values of the expansion factor K as a function of test temperature for various materials from which laboratory ware is made. The numerical values of α, the volumetric coefficient of thermal expansion, given in the column headings are parts per million per degree centigrade. In plastics, columns 4 and 5, this coefficient may vary from batch-to-batch, and the values stated are typical, mid-range values. In the text K is defined as

$$K = 1 - \alpha(T-20)$$

TEMP	BAROMETRIC PRESSURE (MM OF HG)				
(°C)	620	640	660	680	700
18.5	1.002390	1.002418	1.002446	1.002473	1.002501
19.0	1.002480	1.002508	1.002536	1.002564	1.002592
19.5	1.002573	1.002601	1.002629	1.002657	1.002685
20.0	1.002669	1.002697	1.002725	1.002753	1.002780
20.5	1.002768	1.002795	1.002823	1.002851	1.002879
21.0	1.002869	1.002897	1.002924	1.002952	1.002980
21.5	1.002973	1.003000	1.003028	1.003056	1.003083
22.0	1.003079	1.003107	1.003134	1.003162	1.003190
22.5	1.003188	1.003216	1.003243	1.003271	1.003298
23.0	1.003299	1.003327	1.003354	1.003382	1.003410
23.5	1.003413	1.003441	1.003468	1.003496	1.003523
24.0	1.003530	1.003557	1.003585	1.003612	1.003640
24.5	1.003649	1.003676	1.003704	1.003731	1.003758
25.0	1.003770	1.003797	1.003825	1.003852	1.003880
25.5	1.003894	1.003921	1.003949	1.003976	1.004003
26.0	1.004020	1.004047	1.004075	1.004102	1.004129
26.5	1.004149	1.004176	1.004203	1.004231	1.004258
27.0	1.004280	1.004307	1.004334	1.004361	1.004389
27.5	1.004413	1.004440	1.004467	1.004495	1.004522
28.0	1.004549	1.004576	1.004603	1.004630	1.004657

TEMP	BAROMETRIC PRESSURE (MM OF HG)				
(°C)	720	740	760	780	800
18.5	1.002529	1.002557	1.002585	1.002613	1.002641
19.0	1.002620	1.002647	1.002675	1.002703	1.002731
19.5	1.002713	1.002740	1.002768	1.002796	1.002824
20.0	1.002808	1.002836	1.002864	1.002892	1.002919
20.5	1.002906	1.002934	1.002962	1.002990	1.003017
21.0	1.003007	1.003035	1.003063	1.003091	1.003118
21.5	1.003111	1.003139	1.003166	1.003194	1.003222
22.0	1.003217	1.003245	1.003272	1.003300	1.003328
22.5	1.003326	1.003353	1.003381	1.003409	1.003436
23.0	1.003437	1.003465	1.003492	1.003520	1.003547
23.5	1.003551	1.003578	1.003606	1.003633	1.003661
24.0	1.003667	1.003695	1.003722	1.003750	1.003777
24.5	1.003786	1.003813	1.003841	1.003868	1.003896
25.0	1.003907	1.003934	1.003962	1.003989	1.004017
25.5	1.004031	1.004058	1.004085	1.004113	1.004140
26.0	1.004157	1.004184	1.004211	1.004239	1.004266
26.5	1.004285	1.004312	1.004340	1.004367	1.004394
27.0	1.004416	1.004443	1.004470	1.004498	1.004525
27.5	1.004549	1.004576	1.004603	1.004631	1.004658
28.0	1.004685	1.004712	1.004739	1.004766	1.004793

TABLE 5: Values of Z (equation 6) as Explained in the Text.

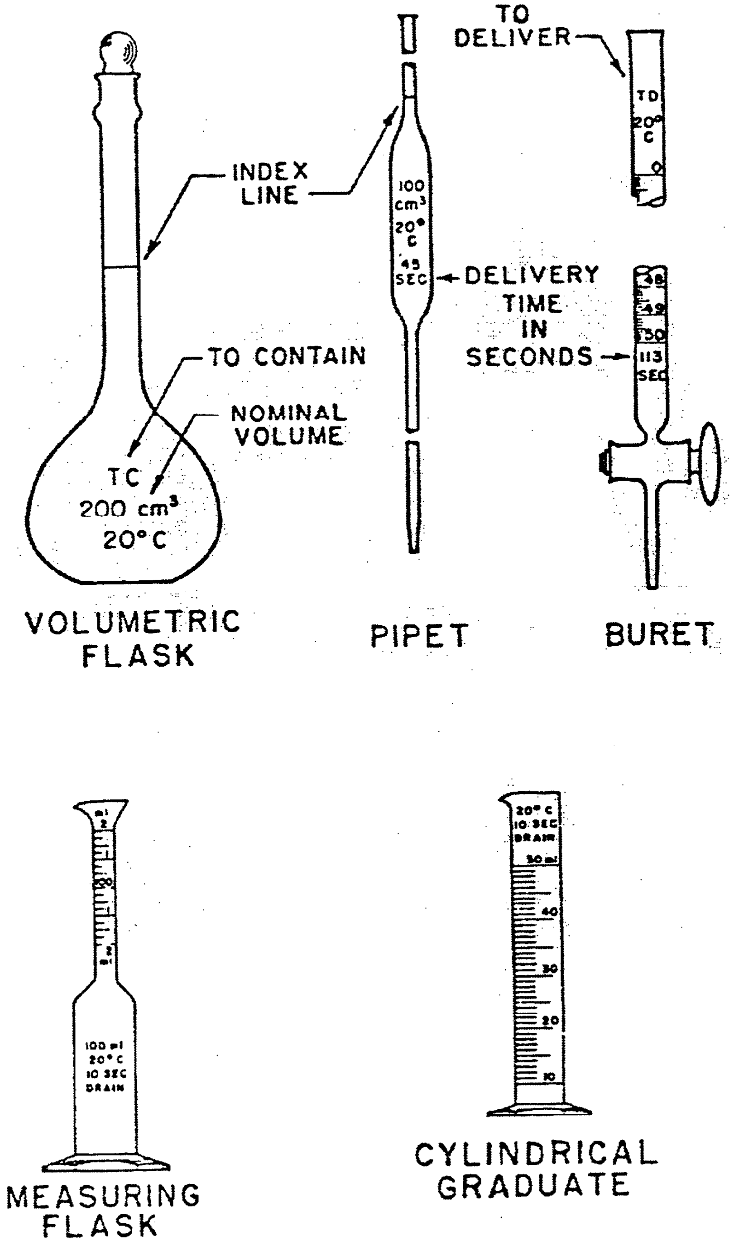


FIGURE 1

SHOWN HERE ARE THE VARIOUS TYPES OF VOLUMETRIC APPARATUS WHICH MAY BE CALIBRATED BY THE TECHNIQUES DESCRIBED IN THIS REPORT.

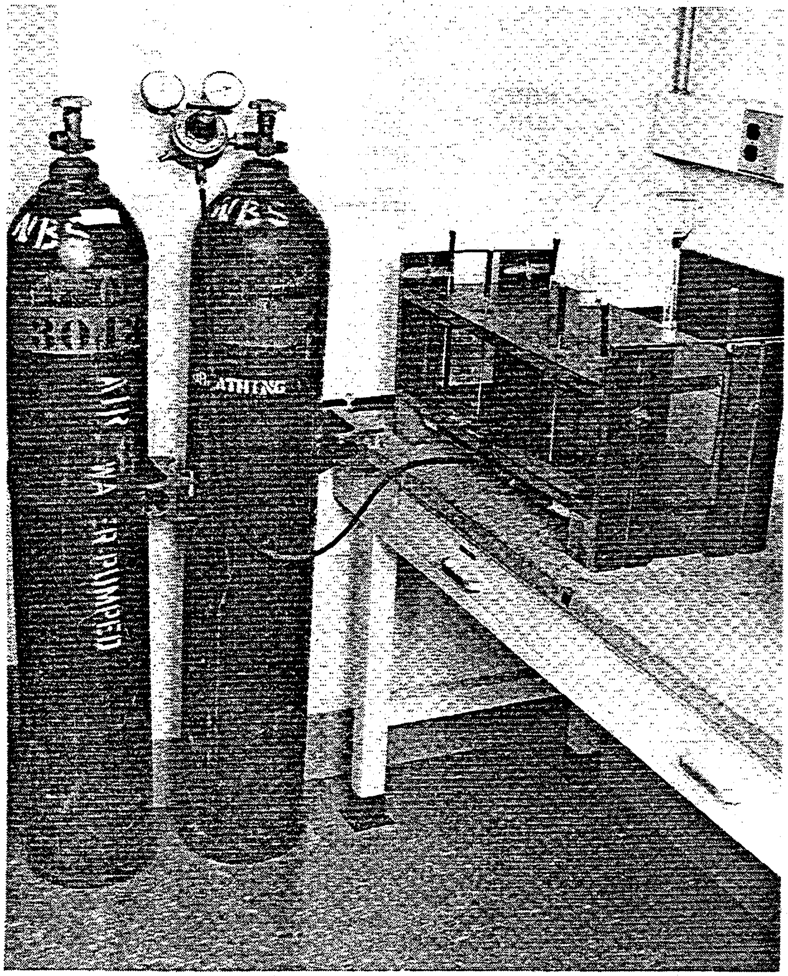
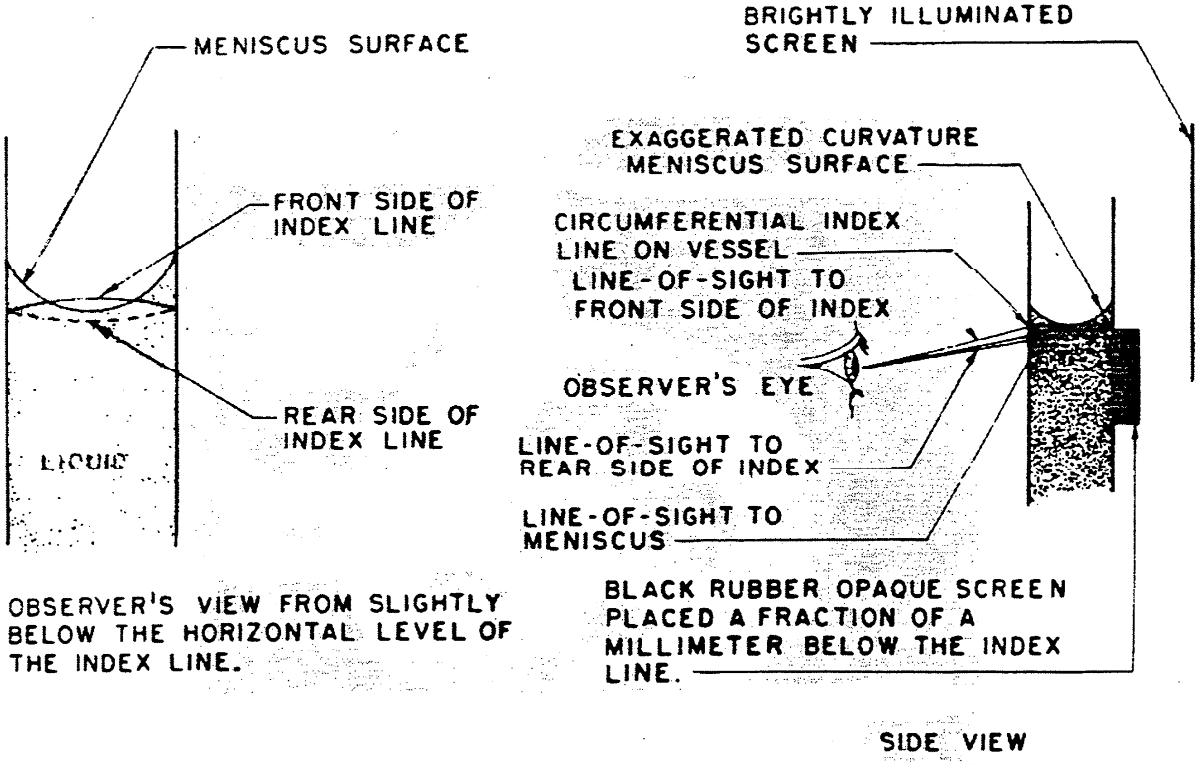


FIGURE 2

DRY AIR BOTTLES AND DRYING RACK
USED TO DRY VOLUMETRIC GLASSWARE

PRESSURE IS ABOUT 1 LB PER SQ IN.

DRYING USUALLY TAKES LESS THAN 10 MINUTES



OBSERVER'S VIEW FROM SLIGHTLY BELOW THE HORIZONTAL LEVEL OF THE INDEX LINE.

SIDE VIEW

FIGURE 3

THESE DIAGRAMS ILLUSTRATE THE PROCEDURE FOR OBSERVING THE MENISCUS POSITION DESCRIBED IN THE TEXT.

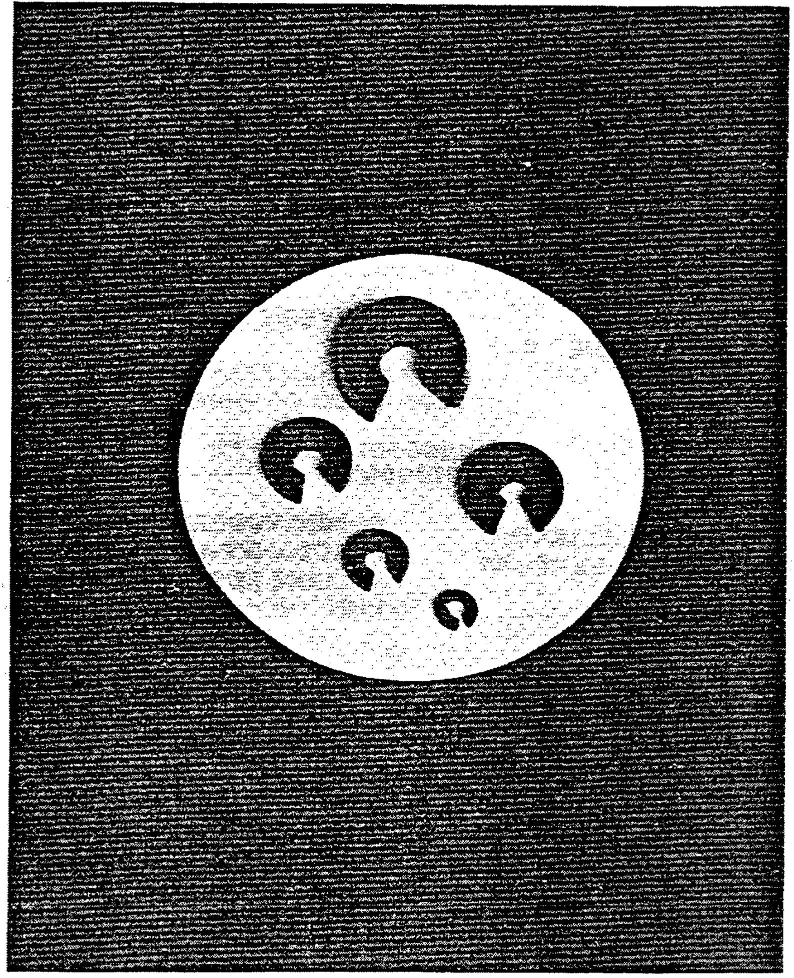


FIGURE 4

VARIOUS RUBBER COLLARS USED TO ASSIST IN THE MENISCUS SETTING DURING THE CALIBRATION OF BURETS, PIPETS AND FLASKS

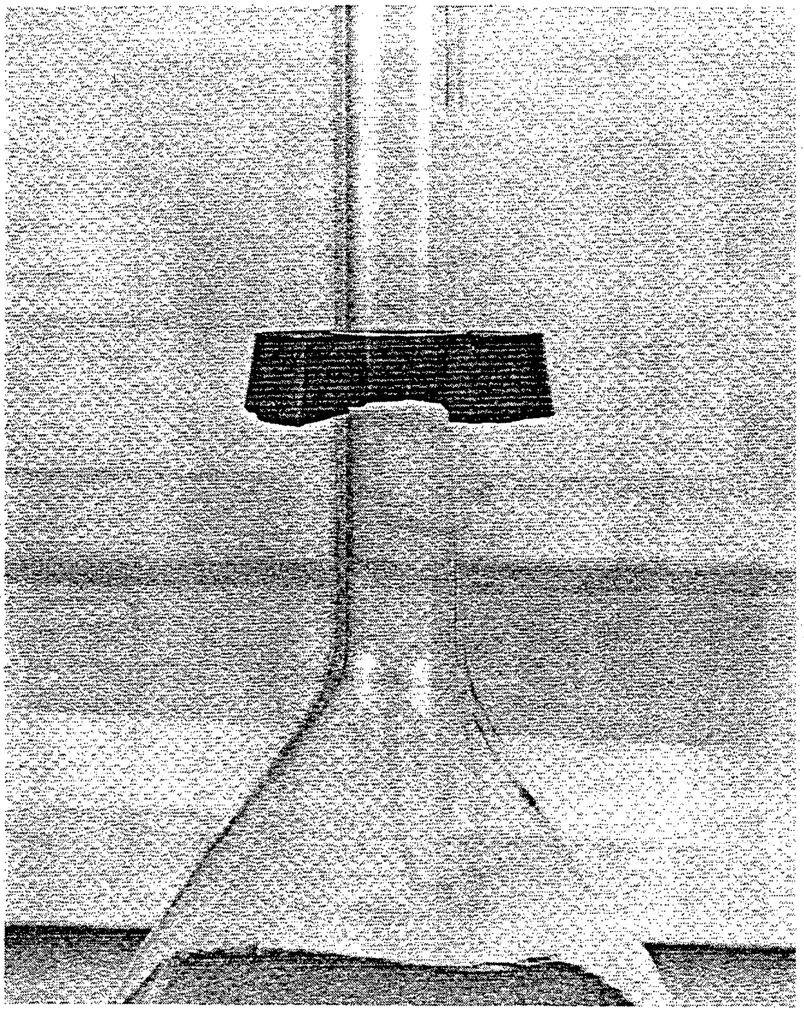


FIGURE 4A

A FLASK WITH A COLLAR-SHAPED PIECE OF BLACK RUBBER TUBING IN PLACE BELOW THE INDEX LINE WHICH RENDERS THE PROFILE OF THE MENISCUS CLEARLY VISIBLE AGAINST A LIGHT BACKGROUND

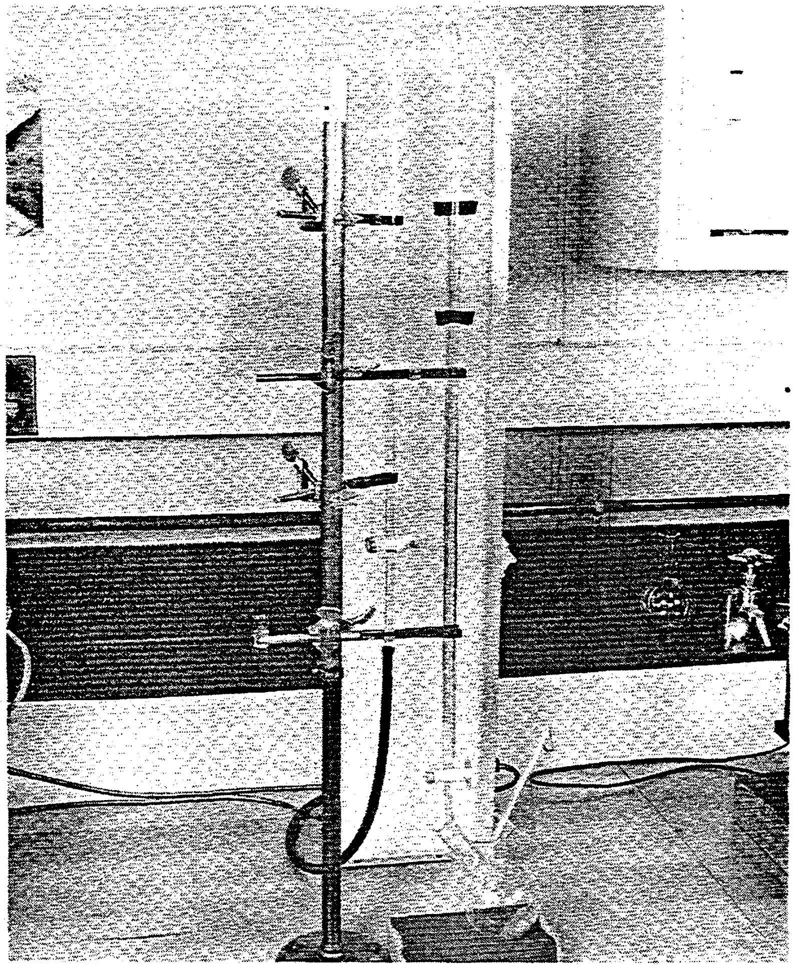


FIGURE 5

APPARATUS USED IN THE CALIBRATION OF A 50ML BURET. THE U-SHAPED TUBE AT THE TOP IS CONNECTED TO A DISTILLED WATER SUPPLY LINE USED TO FILL THE BURET. THE WEIGHING FLASK UNDER THE BURET IS IN POSITION, WITH ITS TIP IN CONTACT WITH THE NECK OF THE FLASK TO RECEIVE THE MEASURED VOLUME. THE BLACK COLLARS AT THE TOP OF THE BURET ARE AN AID IN SETTING THE MENISCUS. THE THERMOMETER IS USED TO OBTAIN A MEASURE OF THE TEMPERATURE OF THE WATER IN THE BURET.

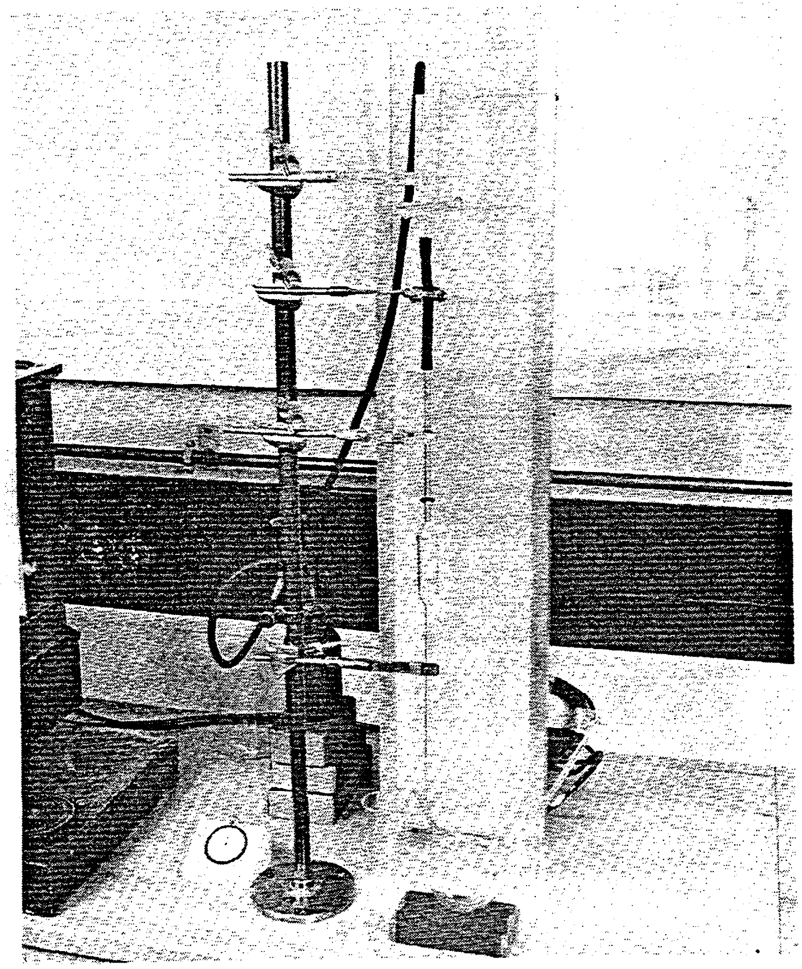


FIGURE 6A

THE PIPET IS DELIVERING ITS MEASURED VOLUME INTO A WEIGHING FLASK, WITH ITS TIP IN CONTACT WITH THE NECK OF THE FLASK. WHEN THE OUTFLOW HAS CEASED THE FLASK IS REMOVED, STOPPERED AND WEIGHED.

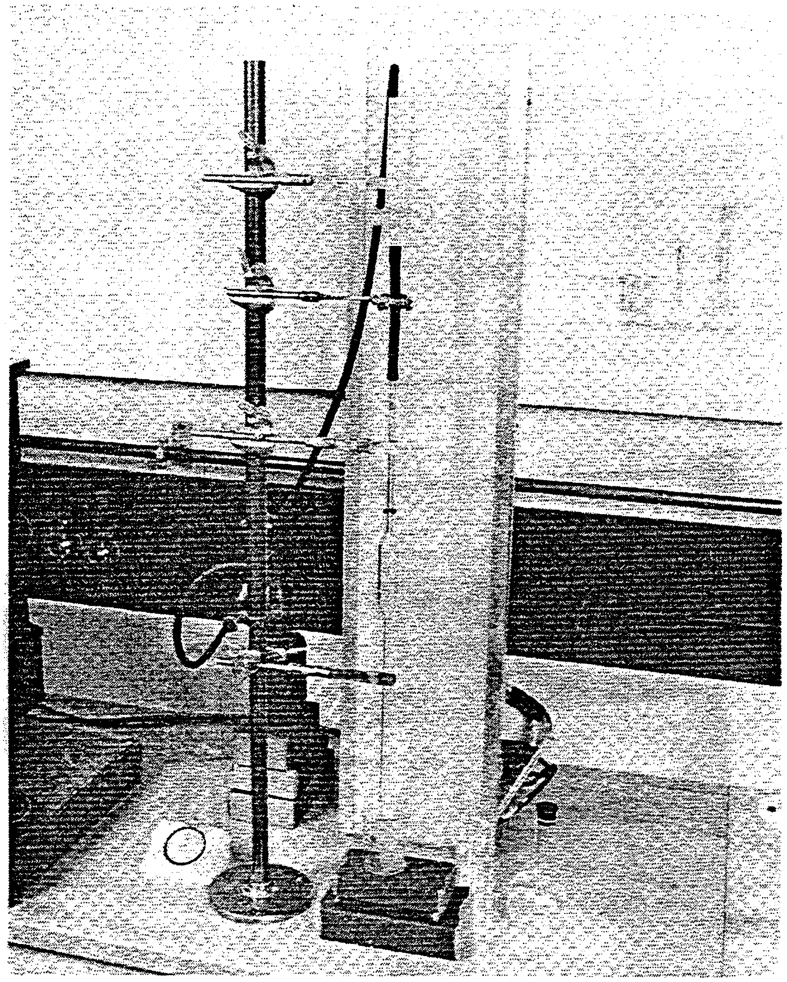
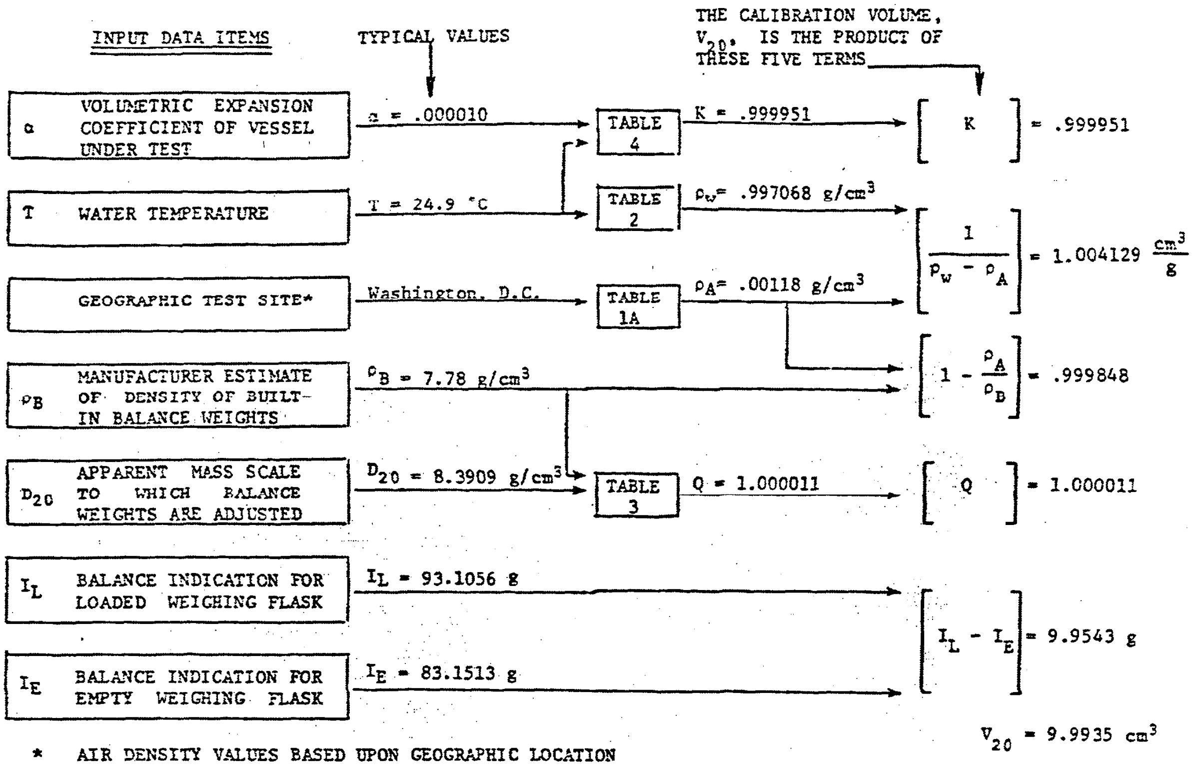


FIGURE 6

TRANSFER PIPET CLAMPED VERTICALLY ON A STAND IN FRONT OF A FROSTED GLASS LIGHT BOX. THE TIP IS IN CONTACT WITH A PIECE OF FROSTED GLASS WHILE THE SETTING OF THE MENISCUS IS MADE. THE HOSE ON THE GLASS TUBE ABOVE THE STOPCOCK IS CONNECTED TO A TRAP BOTTLE WHICH, IN TURN, IS CONNECTED TO THE VACUUM LINE. THE HOSE CLAMP IS USED TO SLOW THE FLOW WHILE MAKING THE MENISCUS SETTING. THE STOPWATCH IS FOR TIMING THE DELIVERY OF THE PIPET. THE TEMPERATURE OF THE DISTILLED WATER IS TAKEN IN THE BEAKER FROM WHICH THE PIPET IS FILLED.



* AIR DENSITY VALUES BASED UPON GEOGRAPHIC LOCATION ARE QUITE ADEQUATE FOR THIS WORK. FOR A BETTER VALUE, TABLE 1B PROVIDES AN ESTIMATE BASED UPON MEASURED TEMPERATURE AND BAROMETRIC PRESSURE AND ASSUMED RELATIVE HUMIDITY.

FIGURE 7

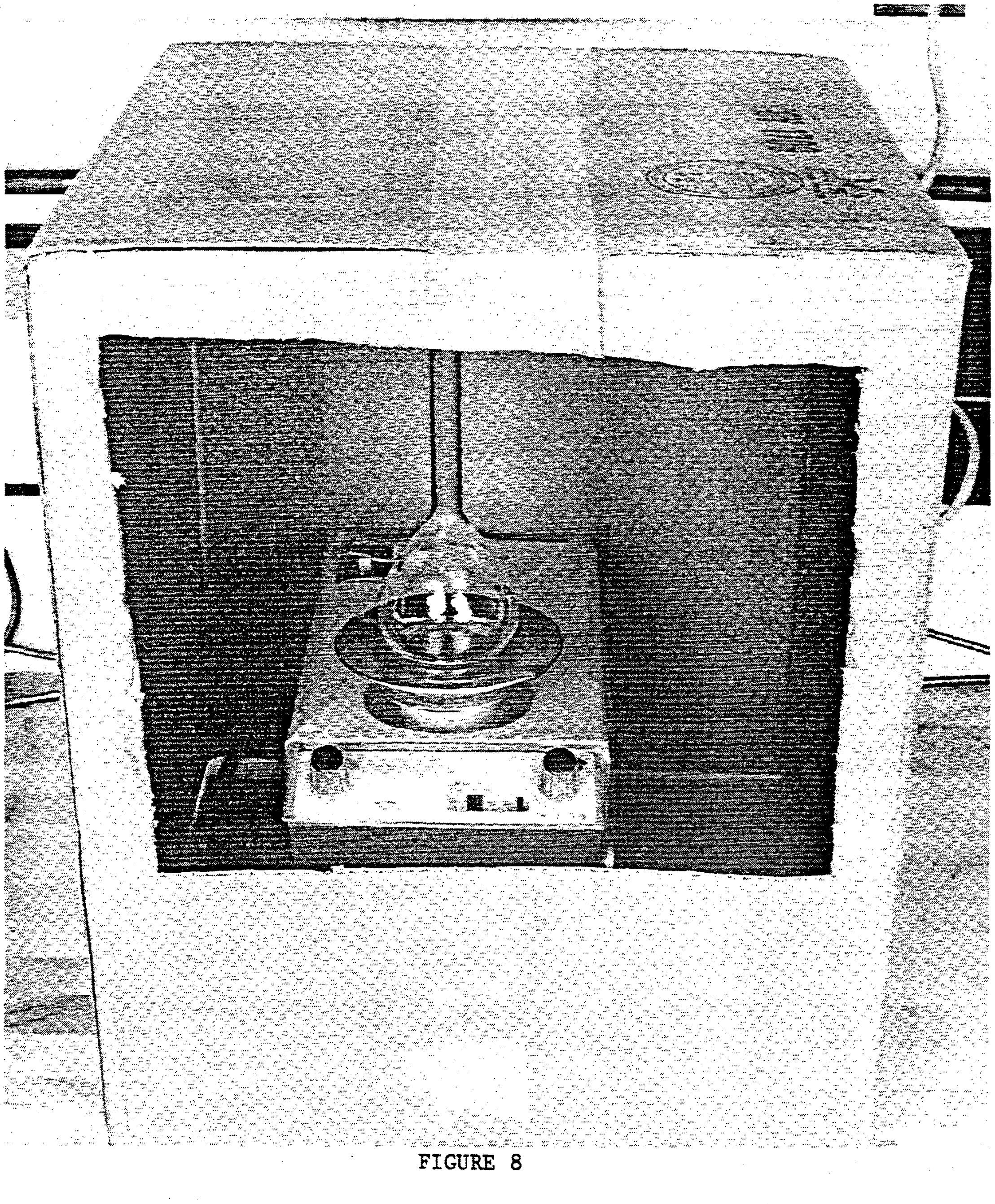


FIGURE 8

1200G TOP-LOADING DIRECT READING BALANCE SUITABLE FOR THE RAPID WEIGHING OF <u>LARGE FLASKS</u>. IT IS SHIELDED BY A CARDBOARD BOX TO PREVENT AIR CURRENTS FROM AFFECTING THE BALANCE READING.

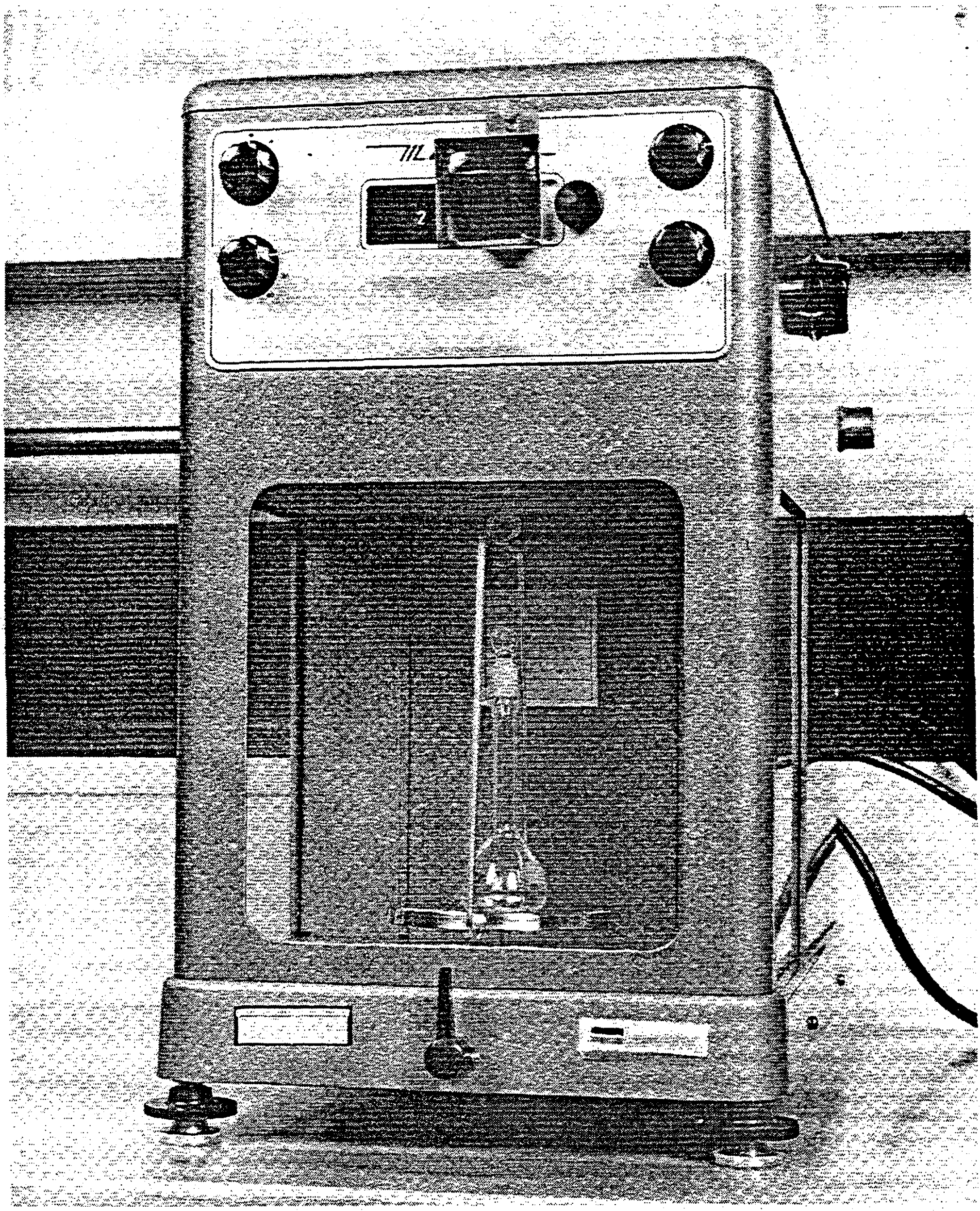


FIGURE 9

100G SINGLE-PAN BALANCE USED TO
WEIGH SMALL <u>TO CONTAIN</u> FLASKS.

U.S. Department of Commerce
Juanita M. Kreps
Secretary
National Bureau of Standards
Ernest Ambler, Acting Director

APPENDIX VII

National Bureau of Standards Certificate

Standard Reference Material 935

Crystalline Potassium Dichromate for Use as an Ultraviolet Absorbance Standard

R. W. Burke and R. Mavrodineanu

This Standard Reference Material consists of crystalline potassium dichromate of established purity. Solutions of known concentrations of this SRM in 0.001 N perchloric acid are certified for their apparent* specific absorbances**, ϵ_a, at 23.5 °C.

This SRM is intended to be used as a reference standard for the verification of the accuracy and linearity of the absorbance scale of absorption spectrometers that can provide an effective spectral bandpass of 1.2 nm or less at 235 nm, and 0.8 nm or less at 257, 313, 345, and 350 nm. Such verification is accomplished by comparing the measured apparent absorbances, A_a, to the A_a calculated from the certified ϵ_a values as described under "Instructions for Use."

Table 1 gives the certified values of ϵ_a in $kg{\cdot}g^{-1}{\cdot}cm^{-1}$ for five concentrations of the SRM 935 potassium dichromate in 0.001 N perchloric acid at 23.5 °C and the indicated wavelengths and spectral bandpasses for a 1-cm internal pathlength.

Table 1. ϵ_a, Apparent Specific Absorbance,[a] $kg{\cdot}g^{-1}{\cdot}cm^{-1}$

Nominal Concentration $g{\cdot}kg^{-1}$	Wavelength and (Bandpass) nm					
	235.0(1.2)	257.0(0.8)	313.0(0.8)	345.0(0.8)[b]	350.0(0.8)	Uncertainty[c]
0.020	12.260	14.262	4.805	10.604	10.672	± 0.034
.040	12.304	14.318	4.811	10.603	10.682	± .020[d]
.060	12.347	14.374	4.816	10.602	10.692	± .020[d]
.080	12.390	14.430	4.821	10.601	10.701	± .020[d]
.100	12.434	14.486	4.827	10.600	10.711	± .020[d]

[a] ϵ_a values are given to the third decimal place to preserve the smooth variation of the data with concentration, although the uncertainties are in the second decimal place.

[b] Wavelength 345.0 nm is near one of the two isosbestic points in $HCrO_4^-/Cr_2O_7^=$ spectra. Because it is on the slope of the composite spectrum, reproduction of the ϵ_a values is dependent on wavelength accuracy. Measurements at this wavelength should be made only for verification of the linearity of the absorbance scale.

[c] ϵ_a values are not corrected for the effects of internal multiple reflections within the cuvette, nor have the weights been corrected to vacuum. With these two exceptions, the uncertainties given include all known systematic errors and the 95 percent confidence interval of the mean.

[d] At wavelength 313.0 nm, the uncertainty is reduced to ±0.010.

---

*The term "apparent" is used because no corrections have been applied to the data for the effects of internal multiple reflections within the cuvette or for buoyancy, i.e., the weights used to express concentrations have not been corrected to vacuum. These combined corrections do not exceed 0.2 percent. The specific absorbances are given in reference 1.

**The nomenclature used in this certificate is that recommended by K. D. Mielenz, Anal. Chem. 48, 1093-1094 (1976), which is reproduced in the Appendix of NBS Special Publication 260-54.

Washington, D.C. 20234
June 1, 1977

J. Paul Cali, Chief
Office of Standard Reference Materials

(over)

The overall direction and coordination of the technical measurements leading to this certificate were performed under the joint chairmanship of I. L. Barnes and J. R. DeVoe.

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by R. W. Seward.

We wish to acknowledge: The contributions of K. D. Mielenz, NBS Analytical Chemistry Division, for his many valuable discussions and suggestions, and H. H. Ku, NBS Applied Mathematics Division, for his statistical treatment of the data; the cooperation and early support of the National Institute of General Medical Sciences in the research leading to this SRM; and the encouragement of George N. Bowers, Jr., M.D., Hartford Hospital, Hartford, Conn., and Royden N. Rand, Ph.D., Eastman Kodak Co., Research Laboratories, Rochester, N.Y.

PREPARATION AND CERTIFICATION

The details of the preparation and certification of SRM 935 are provided in NBS Special Publication 260-54, Certification and Use of Acidic Potassium Dichromate Solutions as an Ultraviolet Absorbance Standard [2]. This publication should be referred to every time SRM 935 is to be used. Briefly, the transmittance, T, of the solutions prepared from the undried, as received, material were measured with the NBS Institute for Materials Research high-accuracy transmission spectrometer [3].

The ϵ_a values were calculated for each wavelength using the relation:

$$\epsilon_a = \frac{D_s - D_b}{b \times c} = \frac{A_a}{b \times c} \qquad (1)$$

where:

ϵ_a = apparent specific absorbance
A_a = apparent absorbance
D_s = transmittance density of the sample solution, $-\log_{10}T_s$
D_b = transmittance density of the blank solution, $-\log_{10}T_b$
b = internal cuvette pathlength, cm
c = concentration, by weight, of $K_2Cr_2O_7$ solution, $g \cdot kg^{-1}$

The crystalline potassium dichromate used for SRM 935 is a special lot of analytical reagent grade material obtained from the J. T. Baker Chemical Co., Phillipsburg, N.J.

Assay: A coulometric assay of the purity of the undried material was performed by G. Marinenko of the NBS Analytical Chemistry Division. The purity of SRM 935, expressed as an oxidant, was found to be 99.972 ± 0.005 percent where the uncertainty figure represents the 95 percent confidence interval for the mean based on 11 degrees of freedom. In addition, the material was examind by optical emission spectrometry for trace elemental impurities by J. A. Norris of the NBS Analytical Chemistry Division. The only significant impurities detected were sodium and rubidium. Their concentrations were estimated to be in the range of 0.02 and 0.03 percent, respectively. Drying at 105 °C for 12 hours showed that the surface moisture of this material was less than 0.01 percent.

Stability:

Solutions prepared from SRM 935 in the concentration range indicated in table 1 and made according to the instructions given in NBS SP 260-54 have been found to be stable within the uncertainties given in table 1 for at least six months when stored at room temperature and protected from evaporation and exposure to light.

INSTRUCTIONS FOR USE

The use of SRM 935 as an absorbance standard requires the careful preparation of a series of solutions of known concentrations, c, of the potassium dichromate in 0.001 N perchloric acid. These solutions are transferred to a quartz cuvette of known pathlength, b, and their apparent absorbances measured at wavelengths 235, 257, 313, and 350 nm, using the spectral bandpass requirements given in table 1. The preparation and measurement of these solutions are described in detail in Section 5 of NBS SP 260-54.

The accuracy of the absorbance scale of the spectrometer being tested is ascertained by comparing the measured apparent absorbances, A_a, of a series of 0.001 N perchloric acid solutions containing 0.020 to 0.100 gram $K_2Cr_2O_7$/kg to the A_a values calculated from the certified ϵ_a values. Although the ϵ_a data in table 1 are given for nominal concentrations of 0.020, 0.040, 0.060, 0.080, and 0.100 g $K_2Cr_2O_7$/kg, the ϵ_a values for concentrations between these nominal concentrations can be determined by linear interpolation. Using the appropriate ϵ_a values, the calculated A_a values at 23.5 °C are obtained from the expression:

$$A_a = \epsilon_a \times b \times c \qquad (2)$$

An example of the calculation of A_a for one concentration of $K_2Cr_2O_7$ under a specified set of conditions is shown below. Calculations of A_a for other concentrations and wavelengths are performed in a similar manner.

Conditions: Wavelength = 350 nm, spectral bandpass 0.8 nm or less
$b = 0.9982$ cm
$c = 0.04375$ g·kg^{-1}
$t = 23.5$ °C

From column 6, table 1, the ϵ_a for concentrations of 0.040 and 0.060 g·kg^{-1} are 10.682 and 10.692, respectively. The corresponding ϵ_a for c = 0.04375 g·kg^{-1} is:

$$\epsilon_a = 10.682 + \frac{0.04375 - 0.040}{0.060 - 0.040}(10.692 - 10.682)$$

$$\epsilon_a = 10.682 + 0.0019$$

$$\epsilon_a = 10.684$$

The calculated apparent absorbance, A_a, from equation 2, is:

$$A_a = 10.684 \times 0.9982 \times 0.04375$$

$$A_a = 0.4666$$

The uncertainty, ΔA_a, in the calculated A_a is determined from the combined uncertainties in ϵ_a, b, and c in equation 2, provided no other systematic errors are present. Thus:

$$\Delta A_a = bc\left|\Delta\epsilon_a\right| + \epsilon_a c\left|\Delta b\right| + \epsilon_a b\left|\Delta c\right| \qquad (3)$$

To evaluate ΔA_a, $\Delta\epsilon_a$ is taken from column 7 of table 1 and the Δb and Δc values must be determined experimentally.

In the experiments performed to obtain the ϵ_a values in table 1, the uncertainties for b and c did not exceed 1 part in 10^4 and 2 parts in 10^4, respectively.

The solution of equation 3 gives:

$$\Delta A_a = 1(0.044)\,(0.020) + 10.7(0.044)\,(0.0001) + 10.7(1)\,(0.0000088)$$
$$= 0.0010$$

Thus, the uncertainty of A_a, for the above set of conditions, is ±0.0010.

The correction of the absorbance scale of the absorption spectrometer under test is determined by plotting the differences between A_a measured and A_a calculated as a function of absorbance. A typical plot of such a graph is shown in figure 1. The apparent absorbances measured on this instrument at 350 nm are accurate when the indicated correction is subtracted from the corresponding absorbance scale reading, provided that the conditions of wavelength accuracy, spectral bandpass, and absence of stray light are fulfilled as specified in NBS SP 260-54. Correction curves for wavelengths 235, 257, and 313 nm are obtained in a similar manner.

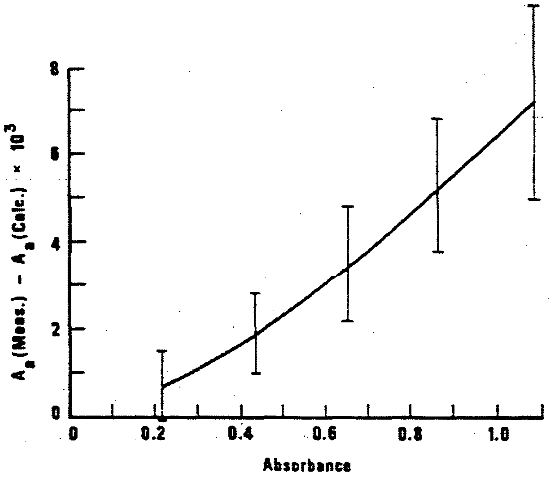


Figure 1. Correction curve for the absorbance scale of a precision spectrometer. The error bars are the sum of the errors arising from the uncertainties in the certified apparent specific absorbances, ϵ_a, cuvette pathlength, b, and concentration, c.

Temperature Correction:

Although ϵ_a values in table 1 are certified at 23.5 °C, SRM 935 can be used as an absorbance standard at other temperatures in the range 20 to 30 °C provided corrections are made to the ϵ_a values. Over this range the apparent specific absorbances decrease linearly with increasing temperature for all the wavelengths given in table 1. The corresponding temperature coefficients, k, for these wavelengths are given in table 2.

Table 2. Variation of ϵ_a with Temperature Over the Range 20 to 30 °C.

λ, nm	Temperature Coefficient, k Percent per degree Celsius
235	−0.05
257	−0.05
313	−0.02
345	−0.08
350	−0.05

The value of ϵ_a at any temperature in the range 20 to 30 °C can be calculated from the certified value and the appropriate temperature coefficient using the relation:

$$\epsilon_a^t = \epsilon_a^{23.5} \left[1 + \frac{k}{100} (t - 23.5) \right]$$

where: ϵ_a^t = apparent specific absorbance at temperature t (°C)

$\epsilon_a^{23.5}$ = apparent specific absorbance certified at 23.5 °C.

k = temperature coefficient, percent per °C.

REFERENCES

1. Burke, R. W., and Mavrodineanu, R., Acidic Potassium Dichromate Solutions as Ultraviolet Absorbance Standards, J. Res. Nat. Bur. Stand. (U.S.), 80A (Phys. and Chem.), No. 4, 631-636 (July-Aug. 1976).
2. Burke, R. W., and Mavrodineanu, R., Certification and Use of Acidic Potassium Dichromate Solutions as an Ultraviolet Absorbance Standard, NBS Spec. Publ. 260-54 (1977). Copies may be obtained from the Office of Standard Reference Materials, National Bureau of Standards, Washington, D.C. 20234.
3. Mavrodineanu, R., An Accurate Spectrophotometer for Measuring the Transmittance of Solid and Liquid Materials, J. Res. Nat. Bur. Stand. (U.S.), 76A (Phys. and Chem.), No. 5, 405-425 (1972).