ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editor

We Learn by Doing

WE WERE greatly disturbed during the recent meeting of the Scientific Apparatus Makers Association to hear of the unfortunate trend in many high schools toward eliminating from physical science courses student participation in laboratory experiments. The staging of certain laboratory demonstrations by the teacher most certainly is part of a well integrated curriculum—experiments that are more elaborate than high school students in most cases can perform personally—but such performances obviously are no substitute for actual participation of the student in supervised experiments.

Apparently there are a number of reasons for the present trend. One, of course, is the readily admitted shortage of teachers adequately trained in the physical sciences. Another is a shortage of money in a number of communities, particularly in some of the most recently developed suburban areas. The third reason, we are told, is a strong belief on the part of certain groups who wield considerable influence that it is more important to impart an appreciation of science in high schools than actually to teach the rudiments.

Joel H. Hildebrand, the outspoken President of the AMERICAN CHEMICAL SOCIETY, on numerous occasions has criticized severely present trends in the teaching of the physical sciences in many of the high schools of the country. Robert E. Wilson, chairman of the board of Standard Oil of Indiana, in his address at the spring meeting of the ACS in Cincinnati, very succinctly summed up present conditions when he stated, "The emphasis appears to be on how to teach, not on what to teach."

It will take more than the eloquence of a Hildebrand and a Wilson to stem the present tide toward mediocrity in our teaching standards. What is needed desperately is the active interest of parents in the type of schooling their children are receiving.

This publication has more than 26,000 subscribers and, perhaps, two or three times as many readers. Many readers have children of high school age. These parents could have a very beneficial effect if they would but exert themselves to influence the thinking in their communities as regards the teaching of science, and to catalyze their nontechnically trained neighbors and friends to undertake positive steps to correct a situation which, if allowed to continue, will have a very detrimental effect on the future welfare of this nation.

A very effective time to begin is when plans for new high schools are being made. This is the critical moment when influence must be brought to bear on school authorities, so that laboratories are provided and not sacrificed for other facilities. These facilities may sound more enticing to those who have no training in the physical sciences and may even be influenced by a positive belief that time spent in studying the physical sciences is largely wasted, but this position cannot be defended.

Through active participation in PTA's, scientists and technologists, including analytical chemists, can see that in the schools already established, sufficient time and attention are given to the teaching of the physical sciences; that school budgets are so prepared that there are adequate provisions for the employment of competent science teachers and purchase of adequate equipment and supplies in order that every student may personally participate in a well rounded program of experiments.

The situation is not so hopeless as it may appear to be. There is a hard core of well trained, loyal, and enthusiastic teachers of physical sciences. Most of these belong to the National Science Teachers Association, affiliated with the National Education Association. Many ACS local sections are cooperating with high school science teachers in their respective areas, particularly with those who specialize in teaching chemistry. Some of these teachers are ACS members. There is reason to believe that eventually the Society will establish some kind of affiliateship for science teachers who do not have the kind of formal training required for ACS membership.

What these devoted men and women need most is our encouragement and assistance. Let us help where we can, but let us do it discreetly and intelligently. The editors of this publication are convinced that our children should be given a proper background in science. This objective can be achieved only if the children themselves perform experiments that have signalized scientific progress down through the years. The true science teachers of this nation believe as we do. Let us help them to get science back to its former position of importance in the high school curricula throughout the length and breadth of this nation. Until this happens, we shall continue to experience shortages of competent scientists. Until this objective is achieved, our populace will not have a true understanding or appreciation of science.

The Importance of Quality Control

IT IS not our intention to add more than a few words to the millions and millions that already have been printed or uttered regarding the most unfortunate turn of events in the polio vaccine program.

Only patience and examination of the vaccines produced by a number of pharmaceutical manufacturers, will disclose if there was anything wrong in the quality of the vaccines produced.

We shall be greatly surprised if the present examination discloses anything faulty with the product manufactured by the six highly responsible concerns. We lean strongly to the belief that those who were stricken after being given the first shot, actually were in an incipient polio stage when the first vaccine was administered. It must also be borne in mind that the statistics released a few weeks ago indicated clearly some polio cases among those children who were treated in the controlled tests of last year.

The point we wish to make concerns the subject of quality control. Top management frequently takes quality control for granted until something dramatic, possibly something tragic, occurs. Then, and only then, do the analytical chemist and the quality control expert get attention, and the attention usually is considerable taking-to-task if the product is not up to grade. Most of the time the man who, day by day, assures the high quality of a finished product in manufacturing operations is the forgotten man.

Analytical Methods and Instrumentation in Air Pollution

Joint symposium of Divisions of Analytical Chemistry, Industrial and Engineering Chemistry, and Water, Sewage, and Sanitation Chemistry in participation with ACS Committee on Air Pollution, presented at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y., September 1954

Introduction

MORRIS KATZ

Defence Research Chemical Laboratories, Ottawa, Ontario, Canada

THE past five years represent a milestone in the application of known and new analytical techniques to the study of air contaminants. The investigations at Donora, Los Angeles, Windsor-Detroit, Cincinnati, and elsewhere have revealed the complex nature of urban pollution and the great range of variability in concentration brought about by meteorological and other factors. The properties, behavior, sampling, and analysis of gaseous and aerosol constituents of the atmosphere representing natural, industrial, and domestic sources have been discussed at a considerable number of technical conferences since 1949. It has become obvious that the final goal of sampling and analytical methods should be the development of continuous, completely automatic instrumentation. An adequate picture of the variation in intensity of gaseous or aerosol contaminants over urban or industrial areas can be obtained only by such continuous techniques. Methods based on intermittent samples collected over relatively short time periods may be useful in locating sources of pollution, but only a continuous technique will yield data that are truly representative of the fluctuating conditions encountered in the field.

PROGRESS IN PAST FIVE YEARS

The contributions to our knowledge of analytical methods and instrumentation in this period may be summarized briefly by reference to a number of symposia and review papers. Important progress has been and will undoubtedly be made in the future through research programs sponsored by the American Society for Testing Materials and other organizations. The work of ASTM Committee D-22 on Methods of Atmospheric Sampling and Analysis, organized in 1951, is expected to provide a series of standardized techniques in this field.

ACS SYMPOSIA

The AMERICAN CHEMICAL SOCIETY has held several symposia on air pollution. At the 115th national meeting in April 1949 (*3*) the papers included methods for the determination of free sulfur and sulfur trioxide in the atmosphere, the use and limitations of the midget impinger, measurement of visibility by photographic photometry, and a discussion of sampling techniques.

At the 123rd meeting of the Society in March 1953 (*2*), there were presented descriptions of two instruments for continuous recording of "oxidant" and fluoride concentrations, respectively. Other discussions dealt with the measurement of dustfall and of atmospheric pollution by ultraviolet photometry. The composition of air with respect to gaseous pollutants as determined by mass spectrometry was also described. The 17th annual Chemical Engineering Symposium on Dispersions in Gases, held in December 1950 (*4*), provided theoretical and research papers of considerable interest to those concerned with air pollution. The properties, behavior, and preparation of liquid and solid aerosols under various conditions were described. Various collection media were discussed, including the performance of dry fibrous air filters, the Venturi scrubber, wet cell washers, and Cottrell precipitators.

XIIth INTERNATIONAL CONGRESS

The International Congress of Pure and Applied Chemistry held a Symposium on Air Pollution in September 1951 in New York under joint sponsorship with the AMERICAN CHEMICAL SOCIETY. This meeting (*12*) provided information on the photochemistry of the lower atmosphere, a discussion of photochemical reactions that may occur in polluted atmospheres, and a description of a procedure for measuring atmospheric fluoride by analysis of rain waters and Spanish moss exposures. A number of papers dealt with the Los Angeles smog problem, such as the chemistry and physiological effects of smog due to the photochemical interaction of oxides of nitrogen with the large quantities of hydrocarbons released in the area; and the contribution of combustion products from the burning of approximately 50,000 tons per day of fuel and refuse, and the exhaust fumes of approximately 2,000,000 automobiles, busses, and trucks in the county. The composition of the organic portion of aerosols in the Los Angeles area as determined by elemental analysis and infrared spectrophotometry was described.

A description was given of a continuous cloud chamber for studying the behavior of foreign particles in the atmosphere, which serve as condensation, freezing, and sublimation nuclei, in relation to precipitation nuclei. The theory of impaction of dust and smoke particles was also discussed.

STANFORD RESEARCH INSTITUTE

Two national symposia on air pollution have been sponsored by the Stanford Research Institute. The first meeting held in November 1949 (*18*) provided useful information on the collection and measurement of aerosols by various methods, including light-scattering and sonic techniques. A continuous recording condensation nucleus meter was described, and various sampling and identification techniques for particulate matter in the atmosphere were discussed. A description was given of the continuous measurement of sulfur dioxide in air by means of the Titrilog analyzer, which absorbs the gas in dilute bromide solution. This type of instrument and the Thomas Autometer are the only two types in general use for the continuous determination of sulfur dioxide in air pollution work.

The second symposium (*18*), held in May 1952, laid stress on the nature, sources, and composition of air contaminants in addition to the toxicological aspects of the problem in relation to community health, animals, and vegetation. Papers were presented on chemical reactions and ozone formation in Los Angeles smog, the nature and composition of blowby and exhaust gases from internal combustion engines, and the products of combustion of gaseous fuels. A useful review was given of the development of instrumentation for the study of air pollution.

U. S. TECHNICAL CONFERENCE

A technical conference on air pollution, one of the largest and most comprehensive held thus far, was convened in Washington at the request of President Truman in May 1950 (*15*). The extremely large number of papers presented at this conference were grouped under seven panels, the following two of which are of interest to the present symposium.

Panel on Instrumentation. This panel was concerned primarily with instruments useful for routine monitoring. The panel considered that continuous sampling with short-time periods is likely to give more valuable information than the long-time average-type sample. Of the fourteen papers presented, four were on the determination of sulfur dioxide and included three distinctly different methods. Papers were presented on electrostatic precipitation, a modified cascade impactor, a recording visibility meter, a sonic method of determining particle size in aerosols, and light-scattering methods of measuring mass concentration and particulate concentration in aerosols.

Panel on Analytical Methods and Properties. The instruments with which this panel was mainly concerned are those used in the laboratory. Papers covered the characteristics of aerosols and methods of determining particle size and number, the changes taking place in air pollutants during dispersion and collection, the difficulties in the analysis and collection of sulfur-containing components, and methods of analysis involving fluorometry, colorimetry, spectroscopy, light spectrography, and infrared techniques.

Recommendations of the panel were that further research could be directed profitably toward the separation and identification of compounds in a multicomponent system, the effects of sunlight and oxygen on atmospheric components in forming noxious compounds, the effect of naturally occurring materials and radiation on discharge gases to form compounds bearing no relation to the source, the physical chemistry of sampling and collection procedures, and the properties of aerosols as related to atmospheric pollution.

MISCELLANEOUS CONFERENCES

Workers who wish to keep abreast of the voluminous literature in this field should consult the proceedings of meetings held by the American Industrial Hygiene Association (*5–7*), the Industrial Hygiene Foundation (*11*), the American Society for Testing Materials (*8*), and the Air Pollution Control Association (*1*). New developments in air pollution research and technology have been covered since 1951 in a monthly column by McCabe in *Industrial and Engineering Chemistry*.

The Symposium on Instrumentation held in May 1954 at the University of Michigan, provided a variety of technical papers on instruments for sampling and analyzing organic vapors in air and for particle sizing, developments in the sampling of air-borne dust, continuous and intermittent sampling devices, filter collecting media, and instruments for filtration of suspended particulate matter in air (*20*).

REVIEW OF PROGRESS

Several papers review the analytical methods and instruments available as a result of progress made in this 5-year period. A fairly comprehensive review of the literature on analytical methods in the postwar period to about 1951 has been presented by Kay (*14*), who points out that new engineering developments and advances in toxicology have focused attention on submicron particles. The important influence of meteorological variables on air pollution has established the necessity for direct-reading continuous instrumentation, for both environmental studies and control work.

Thomas (*19*) reported on the status of instrumentation for the study of air pollution in 1952. He described the operation of the "instantaneous" and "accumulating" autometers for the continuous determination of sulfur dioxide, based on the principle of the change in conductivity of the absorbing solution in contrast to that of the Titrilog analyzer. Other automatic equipment for hydrogen sulfide was also described. Hydrogen fluoride can be determined in the "accumulating" autometer after removal of absorbed sulfur dioxide. Thomas also presented a discussion of two types of oxidant-recording analyzers developed by the Stanford Research Institute, and the freeze-out technique for the collection of hydrocarbons in the atmosphere. Instruments for collection of particulate matter included the directional dust fall collector of Munger, the paper filtration apparatus of Owen and its recent modifications, and the Autosampler of Hall (*10*). Various visibility meters and instruments based on the forward scattering of light for measurement of fine aerosols were also reviewed.

More recent reviews are those of Clayton (*9*) and Katz and Clayton (*13*). These papers discuss sampling equipment for particulates based on filtration technique, such as the high volume air sampler, continuous filter paper samplers of the Hall Chaney, Wilson, and Hemeon types, and the Transmissometer for measurement of visibility. More conventional aerosol collection equipment, including the thermal and electrostatic precipitators, the cascade impactor, and a spiral sampler, is also described. Both papers include a review of methods and instruments for the analysis of various gases. Clayton has provided a useful tabulated summary of analytical methods and equipment used for determining common air pollutants.

THE PRESENT SYMPOSIUM

This symposium consists of five papers; four deal with various studies of fine aerosols and one is concerned with course particulates.

O'Konski and Doyle of the University of California, Berkeley have developed a versatile light-scattering instrument of high sensitivity, which employs the right-angle scattering system. Last year, Sinclair (*17*) described the design and operation of an instrument employing forward scattering of light for measuring the mass concentration of suspended particulates in the atmosphere, in conjunction with a recording potentiometer circuit. The present instrument represents another important achievement in this field of submicron particles.

The principle of measurement of light transmittance through a long path has been employed in the Transmissometer used by Clayton and Giever to study visibility as influenced by

)ollution in Detroit. This instrument was developed by C. A. Douglas of the National Bureau of Standards and was designed o aid in flying safety by eliminating or reducing the errors introduced in visual estimations by the human factor.

Clayton and Giever have compared this transmittance method vith filtration methods for aerosols, such as the determination of haze and smoke concentrations by reflectance of spots on paper and the weight of air-borne solids collected by the high volume sampler.

A microanalytical technique for the determination of sulfuric acid aerosol has been developed by Gerhard and Johnstone of the University of Illinois as a result of studies on the rate of the photochemical oxidation of sulfur dioxide.

The use of microscopic and spot test reactions in the identification of particulate air pollutants is discussed by Monkman of he Occupational Health Laboratory, Ottawa, Canada. The identification of a number of inorganic compounds in air-borne articulates by electron microscopy and x-ray diffraction was escribed recently by Shore and Katz (*16*).

A comparison of cylindrical glass jars, partially filled with vater, and greased plates in the measurement of dustfall has een made by Pond and Paxton of Stanford University. The reased plates yielded more reproducible results and gave slightly igher rates of dustfall.

SUMMARY

Notable progress has been made in the development of new ıethods and instruments for both gases and aerosols. Perhaps ıe greatest advance in this 5-year period has been in the aplication of the fruits of theoretical research during and after ıe war on the physical and chemical properties of aerosols. lowever, much more information is still required on specific ıalytical and identification techniques. Virtually no attempt as been made to apply polarographic technique to air pollution udies, such as the estimation of hydrogen fluoride, sulfur diide, and other gases. There is room for improvement in availble methods for the determination of low concentrations of ides of nitrogen, and a continuous analyzer is badly needed.

A considerable amount of information has been accumulated on the composition of the inorganic fraction of aerosol contaminants by x-ray diffraction, spectrochemical, and microchemical analysis. Little is known, however, about the composition of the organic fraction, which is extremely complex. The best approach to this problem probably lies in chromatographic adsorption methods of separation, followed by examination of the isolated material by ultraviolet and infrared absorption. Where isolated fractions of organic material show fairly sharp and specific absorption bands, microchemical and spot test reactions will aid in identification.

LITERATURE CITED

(1) Air Pollution Control Assoc., *Air Repair*, **3**, 3–56 (1953).
(2) Am. Chem. Soc., Abstracts of Papers of 123rd Meeting, March 1953.
(3) Am. Chem. Soc., *Ind. Eng. Chem.*, **41**, 2384–93 (1949).
(4) *Ibid.*, **43**, 1312–80 (1951).
(5) *Am. Ind. Hyg. Assoc. Quart.*, **11**, 7 (1950).
(6) *Ibid.*, **13**, No. 4 (1952).
(7) *Ibid.*, **15**, No. 4 (1954).
(8) Am. Soc. Testing Materials, Committee D-22, *Am. Soc. Testing Materials Proc.*, **53**, 485 (1953).
(9) Clayton, G. D., "Determination of Atmospheric Contaminants," Am. Gas Assoc., **PC-53-12** (1953).
(10) Hall, S. R., Anal. Chem., **24**, 996 (1952).
(11) Ind. Hyg. Foundation Am., *Trans. Bull.* **13** (1949).
(12) Intern. Congr. Pure Appl. Chem., *Ind. Eng. Chem.*, **44**, 1339–88 (1952).
(13) Katz, M., and Clayton, G. D., *Am. Soc. Testing Materials Proc.*, **53**, 1136 (1953).
(14) Kay, Kingsley, *Ind. Eng. Chem.*, **44**, 1383 (1952).
(15) McCabe, L., chairman, "Proceedings of U. S. Technical Conference on Air Pollution," McGraw-Hill, New York, 1952.
(16) Shore, Violet, and Katz, M., *Am. Ind. Hyg. Assoc. Quart.*, **15**, No. 4, 297 (1954).
(17) Sinclair, D., *Air Repair*, **3**, 51 (1953).
(18) Stanford Research Inst., Pasadena, Calif., Proceedings of First National Air Pollution Symposium, 1949; Proceedings of Second National Air Pollution Symposium, 1952.
(19) Thomas, M. D., *Ibid.*, pp. 16–23.
(20) University of Michigan, Symposium on Instrumentation, Anal. Chem., **26**, 28A (March 1954). Titles only.

Received for review September 3, 1954. Accepted March 17, 1955. Contributed as DRCL Report No. 192.

Light-Scattering Studies in Aerosols with a New Counter-Photometer

CHESTER T. O'KONSKI and GEORGE J. DOYLE

Department of Chemistry and Chemical Engineering, University of California, Berkeley, Calif.

The functions of a recording light-scattering photometer and electronic particle counter were combined in a single instrument, employing an improved right-angle optical system and a single electron multiplier phototube. Techniques were developed for standardization of the instrument by light scattered from filtered gases. As expected from theoretical considerations, the intensities from helium, hydrogen, nitrogen, carbon dioxide, sulfur dioxide, and methyl chloride increased approximately with the square of the mean molecular polarizabilities. The limit of sensitivity of the photometer was 3×10^{-11} gram per liter of a test aerosol 0.3 micron in diameter. Procedures were developed for producing uniform aerosols from uniform hydrosols. Pulse amplitudes of test aerosols 0.33, 0.5, and 1 micron in diameter obeyed the remarkably simple law of being proportional to the square of the particle diameters. The resolving power of the counter lies within a standard deviation of 8% in diameter, and the lower limit of sensitivity for single particles is a diameter of 0.3 micron.

LIGHT-scattering measurements on aerosols have been used intensively since 1942, and are well established for the determination of relative concentrations (*15*) under conditions such that the particle size distribution of the aerosol remains unchanged. Sensitive instruments, such as the one developed at Northwestern University in 1943 (*16*), were capable of detecting 10^{-9} gram per liter, or around 70 particles per cc., of an oil smoke consisting of droplets around 0.3 micron in diameter. For particles above 0.6 micron in diameter, much greater sensitivity was achieved with the development of the first instrument capable of electronically counting the aerosol particles

(*12*, *14*) individually. Subsequent refinements of this instrument (*13*, *17*) were directed toward the determination of size distribution in aerosol systems. At the extremely low concentrations necessary for counting particles separately, experimental difficulties encountered in producing and maintaining aerosols of very uniform and accurately known particle size precluded adequate testing of the refined models.

Recently, there have been produced (Physical Research Laboratory, Dow Chemical Co., Midland, Mich.) uniform spherical particles of polystyrene and polyvinyltoluene in the size range from 0.1 to 1 micron. Such particles, when dispersed individually as an aerosol, are ideal for calibration and evaluation of counting instruments because of their high degree of uniformity and low vapor pressures. Results on several of the uniform preparations, which establish that the light-scattering particle counter is feasible for the rapid determination of size distribution in aerosol systems, are presented here.

The present instrument is both a refinement and a combination of previous photometers and counters.

LIGHT-SCATTERING INSTRUMENT

A schematic diagram of the apparatus is shown in Figure 1.

Aerosol particles in a suitably defined stream, perpendicular to the plane of the diagram, are passed through the region designated by the dark spot at the intersection of the dashed lines. This region is intensely illuminated by means of the light source, *S*, in conjunction with the lenses, *L*1 and *L*2, the aperture, *S*1, and the field stop, *S*2. Particles entering it scatter a small fraction of the incident light. The scattered light within a large solid angle in the vicinity of 90° is collected by the lenses, *L*3, and brought to focus in the plane of the field stop, *S*9, from which it proceeds to the cathode of a photomultiplier tube, *P*. As the particles traverse the illuminated region in a period of the order of a millisecond, the electrical signals at the photomultiplier appear in the form of pulses, with amplitudes which depend upon the flux reaching the photocathode. The pulses are sent to discriminator and counter circuits for pulse amplitude distribution analysis and the direct current component of the photomultiplier current is presented on a recorder.

The optical system was chosen after considerable experimentation with various arrangements. Both small-angle and right-angle light-collecting systems were set up. Various glare stops and especially designed knife-edged light traps were tested. The right-angle system proved to have a greater sensitivity for submicron particles than the small-angle arrangement which was tested, and it was adopted for this work. In its final form, there were several modifications from the earlier instrument (*13*), which include insertion of various additional stops, *S*3 to *S*8, and reduction of the dimensions of the slits and the diameters of the aerosol and sheath-air tubes. By these means, the background flux to the photomultiplier detector was reduced, which resulted in a decrease of the random noise and an increase of the sensitivity and precision for weak signals.

Field stops *S*2 and *S*9 were rectangular 0.10 × 0.20 cm. slits. The longer dimensions were transverse to the aerosol tube axis. The internal diameter of the aerosol tube was 0.15 cm. The total scattering volume, v_t, was 0.004 cc., and the sensing volume, v_s, was 0.0017 cc.

The total scattering volume is defined as that volume which is both illuminated by the source and viewed by the photomultiplier. For comparison of various right-angle optical systems, it can be calculated as the product of the slit (*S*2) dimension, measured transversely to the direction of the aerosol tube axis, the slit (*S*9) dimension, similarly transverse, and the smaller of the two remaining dimensions of *S*2 and *S*9. This assumes rectangular slits and neglects the effect of the slight spreading at the focal region due to convergence. The sensing volume is defined as the volume of the aerosol stream both illuminated and viewed. As the transverse dimensions of *S*2 and *S*9 completely cover the stream, so that all particles are counted, it can be calculated as the product of the area of the aerosol stream at the focal region, and the third dimension employed in the calculation of tota scattering volume. With the previous right-angle optical sys tem (*13*), v_t and v_s were about 0.016 and 0.008 cc., respectively This situation was not properly evaluated in previous work (*13*)

The photoelectric detector, *P*, was the better of two RC/ 1P21 photomultipliers. Both tubes had somewhat higher signal to-noise ratios in this application than any of the earlier 931 an 931-A tubes (*13*), some of which were retested in this researcl The regulated high-voltage power supply was a circuit of th Higinbotham type (*22*). The photomultiplier dynode voltage were obtained from a resistance divider network. The loa impedance was 10^7 ohms shunted by the dynamically measure distributed capacity, 21.5 μfd., which decreased the noise leve by suppressing high-frequency response. The photomultiplie was direct-coupled to a differential cathode-follower amplifier (*7*)

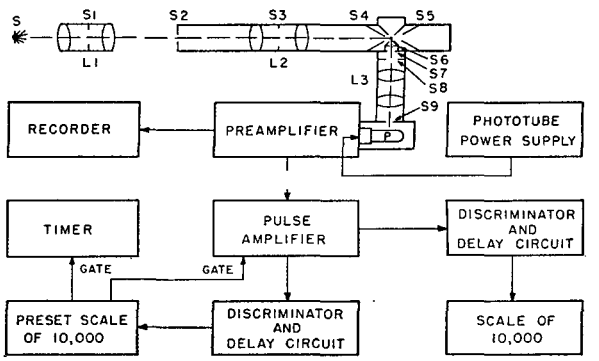


Figure 1. Schematic diagram of light-scattering counter-photometer

The direct current signal was filtered and applied through a variable attenuating circuit to a 2.5-mv. Brown potentiometer-type recorder. Suitable zero-balance and measuring circuits were incorporated into the direct current network, which was used for the photometric measurements, and precautions were taken to ensure high stability. The output current of the photomultiplier could be measured potentiometrically by means of a Helipot.

The alternating current signal from one side of the differential amplifier was increased about 100-fold in the capacitively coupled feedback amplifier, which employed compensated networks to ensure faithful reproduction of pulses. The amplifier output stage was a gated cathode follower. The gate signal was derived from a preset scaler, and turned off the output stage after a predetermined number of counts. The differential amplifier was so arranged that signals were clipped at about 2 volts, to prevent blocking of the amplifier and the discriminator circuits on relatively large pulses.

The amplified signals were fed to electronic discriminators of the Schmitt type (*7*). Each discriminator provided a rectangular output pulse whenever the signal exceeded a bias level, which was adjusted between 0 and 100 volts by means of a Helipot. The discriminators were capacitively coupled to the cathode follower output stage of the amplifier, and diode clamping circuits were inserted at the grids to ensure rapid recovery after large pulses, and to maintain the proper reference level at higher counting rates.

The output from each discriminator was passed through a differentiating circuit and into a univibrator. This unit was so arranged that, upon being triggered, it was not affected by a second pulse occurring within an interval, T_d, of the one preceding. The delay time, T_d, was made slightly longer than T_a, the duration of the pulse from an aerosol particle. This provided an electrical delay which ensured that pulses in coincidence would always be recorded as one pulse, and that multiple counting of individual pulses could not occur. It can readily be shown that, because of the random noise, pulses with amplitudes near the discriminator bias level would at times be counted more than once, unless some sort of electrical delay were incorporated. This was verified by experiment.

The large output pulses from the univibrators, or delay circuits, were differentiated and fed to corresponding scalers, which consisted of decade units of the modified binary type (Berkeley Scientific Division, Beckman Instruments Co., Richmond, Calif.). The simple decades were Type 700A, and the preset decades, Type 730. These units have a resolving time of 5 microseconds.

Two scales of 10,000 were assembled. One of the scaling circuits contained two preset decades, so that after an experiment had begun, counting would proceed through the interval corresponding to any preset integral number, between 1 and 99, of hundreds of counts on this scaler. After this interval a gate signal was automatically applied to the amplifier, which stopped all counting, and to a relay circuit, which controlled a timer. Appropriate circuitry was incorporated to reset the scalers and timer electrically. All power supplies were electronically regulated wherever the voltages affected the operation of the discriminators, and quality components were employed for the critical circuit elements. In the differential preamplifier circuit, where the current requirements were low and extreme stability was desired, two small batteries were employed.

EXPERIMENTAL PROCEDURES

Production of Aerosols. Aerosols were generated from hydrosols by the spray-dry technique employed in previous work (*9, 13, 14*).

A Vaponephrin (*12*) nebulizer was employed with a primary air pressure of 5 pounds per square inch, and 30 liters per minute of diluting air. The primary air stream was humidified to reduce evaporation losses and resulting concentration changes in the hydrosol. The diluting air stream was predried in a calcium chloride tower. Both air streams were filtered with multiple pads of asbestos-filled paper.

Latex Preparations. Experimental samples of polyvinyltoluene and polystyrene latex hydrosols were supplied by the Dow Chemical Co. The properties of the six samples received are summarized in Table I. The first five columns contain the Dow run number, the composition, the mean diameter (from electron micrographs), the standard deviation, and the number of particles, from which the statistical data were obtained by the Dow laboratories. The last two columns contain the weight concentrations of latex and stabilizer, determined in this laboratory by a straightforward gravimetric technique, involving centrifugation of the latex particles and evaporation of the remaining solution. The composition of the stabilizer employed was not given.

Table I. Properties of Latex Hydrosols

Run No.	Composition[a]	Mean Diameter, Micron	Standard Deviation, %	No. of Particles	Latex, G./Cc.	Stabilizer, Mg./Cc.
44-D	PVT	0.986	1.6	21	0.106	5
15N-8	PS	0.514	2.1	209	0.110	4
44-A	PVT	0.470	1.1	29	0.266	5
15N-7	PS	0.333	2.1	285	0.102	4
40	PVT	0.144	2.8	76	0.186	6
15N-23	PS	0.132	6.8	447	...	..

[a] PVT = polyvinyltoluene; PS = polystyrene.

Each sample was diluted to a particulate concentration of about 10^8 per cc., to reduce the aerosol concentration to an appropriate level, and to improve uniformity.

Light Sources. The sources in this work were the ribbon-filament tungsten lamp, and the 100-watt concentrated arc (*13*). The tungsten source was employed with an image-to-object ratio of unity, but the concentrated arc was used with a linear ratio of about 2, to cover adequately the field stop, *S2*. Operation was at the manufacturer's rated values.

Sampling and Optics. The aerosol sample was taken from the open end of the spray-dry generator through a short length of Tygon tubing to the central aerosol tube. The flow rate was set at 100 cc. per minute by maintaining this difference between the exhaust and sheath-air flow rates, employing the flow system described in earlier work (*13, 14*). As the test aerosols used here were relatively uniform and in the submicron region, fractionation was not a problem, and special precautions were not required.

The field stop dimensions are given above. The half-angle of the scattered light-collecting lens system was 15.6°. That of the illuminating system, determined by aperture *S1* of Figure 1, was 12.2° with the tungsten source, and 10.8° with the concentrated arc source. The pulse length estimated for these conditions, assuming uniform flow of the aerosol through the cross section of the stream, was 1.06 milliseconds; that experimentally observed with a triggered oscilloscope was 1.0 millisecond.

Photometric Measurements. Because the sensing volume, defined above, normally depends upon the flow rates, the measurements on gases were made after the entire cell had been flushed with the carefully filtered gases. This results in improved precision, because the total scattering volume is a function of the optical dimensions only. An alternative procedure would be to arrange the aerosol stream diameter and the stop dimensions in such a manner that, allowing for extreme variations in the flow rates, the sensing volume would always be filled completely with the stream being measured. This would make the sensitivity independent of flow rates, and would utilize the sheath-air system to prevent mixing of the aerosol with the contents of the cell, and flushing time could be reduced to a fraction of a second.

After the photomultiplier voltage and the attenuating circuit had been adjusted to appropriate levels, generally ascertained by a preliminary experiment, the deflections of the recorder were observed, first with the illumination on, and then cut off by a shutter. The difference in deflections was a measure of the photocurrent, excluding dark current. By operating the recorder during the flushing operations, it was conveniently observed when the deflection reached a steady value, which required about 2 minutes.

The relative gain of the photomultiplier was measured for voltages from 41.6 to 125 per stage. The gain curve was slightly steeper than that for an average 1P21 (*25*). At the relative gain of 1.00 (55.5 volts per stage) the average 1P21 has a current amplification of 3.5×10^4.

Counting Procedures. Because coincidences contribute relatively large pulses, even when the aerosol particles are uniform, it is desirable to reduce the fraction of coincidences to a negligible value. The number of coincidences of two particles within the sensing volume, v_s, can be reduced to a small fraction of the number of particles if the aerosol particulate concentration, n_a, is adjusted to a value such that $n_a v_s << 1$. Treating the occurrence of a second particle within v_s as a random event, the fraction of coincidences is then simply $n_a v_s$. An alternative and equivalent expression of the fraction of coincidences, when the pulses are all of equal length, is $T_p r$, where T_p is the duration of a pulse, 0.001 second, and r is the count rate per second. Accordingly, the concentration was adjusted in each experiment until $T_p r$ equaled the desired coincidence level. In the experi-

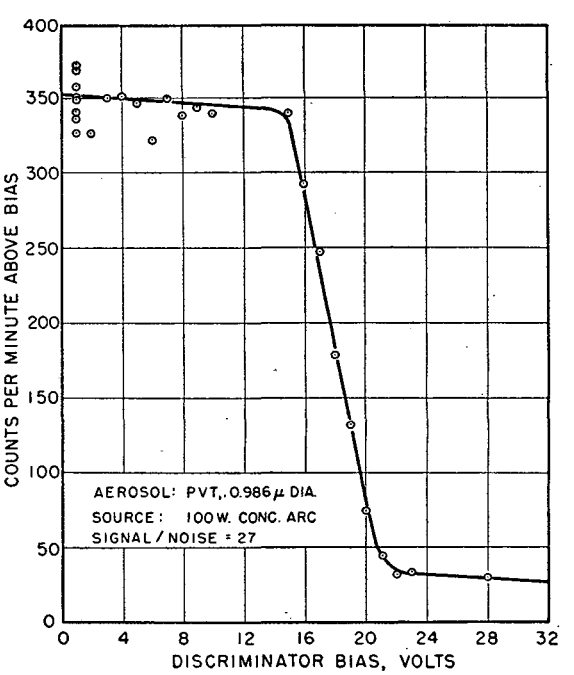


Figure 2. Integral count rate curve (experiment 12)

ments reported below, this was always less than 2% of the total count rate.

The photomultiplier gain was adjusted to a level such that the mean pulse amplitude was between 10 and 50 volts at the discriminators. For detection of signals which were just above the noise, it was desirable to have the noise level 10 to 100 times the limit of precision of the discriminator circuits, which was found to be 0.1 volt. When necessary, the background rates due to random noise were subtracted from the observed rates at each discriminator setting, and corrections for the dead time in the circuit were applied.

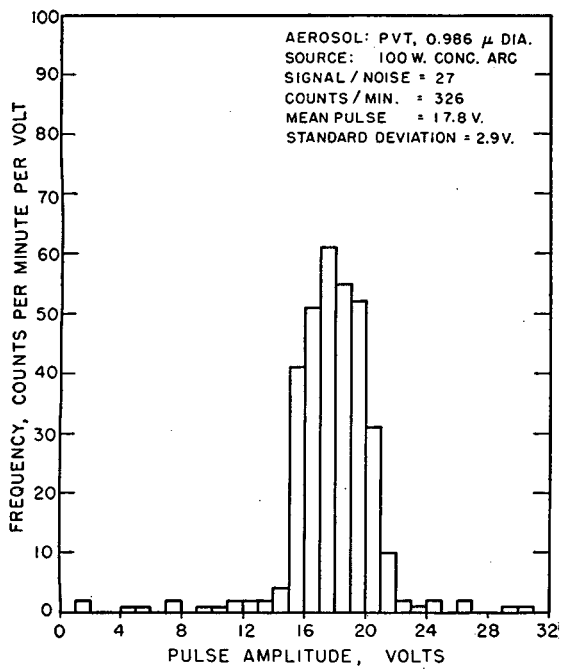


Figure 3. Pulse amplitude distribution (from Figure 2)

Working Definition of Noise Level. In previous work (*13*), the amplification in the electronic system was adjusted until the average noise level reached an arbitrary predetermined value. For computation of signal-to-noise ratios in this work, the noise level is defined as that setting of the discriminator at which the random noise fluctuations, produced by the steady background flux with air in the cell, resulted in 300 counts per minute. This definition, which is by necessity arbitrary, was convenient because the noise level could be measured accurately with the counting equipment.

EXPERIMENTAL RESULTS

Oscillographic Experiments. Preliminary experiments involving photometry and oscillographic photography were conducted with an optical system essentially identical to that previously used (*13*), except for the size of slits *S*2 and *S*9. It was found that the ratio of stray light to air scattering from the total scattering volume was 1.3, or about four times that of the previous optical system. Considering the fact that there was a fourfold decrease in total scattering volume, this indicated that the absolute stray light level was practically unchanged by reduction of slit dimensions. In the same experiments it was established that the instrument gave essentially equal response ratios to particles 0.986 and 0.470 micron in diameter when used as a counter (1.00 to 0.23), or as a photometer (1.00 to 0.24) at equal particulate concentrations.

Subsequently stops *S*4 and *S*8 were inserted, and stray light was decreased to 0.4 of the scattering from air, or 28% of the total background with air in the cell. Again considering the volume ratio, this means about a fourfold reduction in the stray light compared to the earlier right-angle instrument.

Table II. Comparison of Mean Pulse Heights from Latex Aerosol Particles

Expt.	D	Latex	Source	S/N	$10^9\ i_p$	σ, %	K
12	0.986	PVT	Zr	27	8.4	16	9.3
53	0.514	PS	Zr	7.3	2.24	14	9.2
54	0.333	PS	Zr	3.3	0.97	11	9.4
11	0.986	PVT	W	8.4	7.9	28	9.0
61	0.986	PVT	W	13.5	7.8	16	9.0
61	0.514	PS	W	3.6	1.92	14	8.5
60	0.514	PS	W	3.4	1.93	13	8.6
60	0.333	PS	W	1.8	0.92	15	9.1
59	0.333	PS	W	1.6	0.88	15	8.9
						Av.	9.0
						Mean deviation	0.2

Table III. Photometric Measurements on Gases

Gas	$10^9\ i$	$10^9\ i_g$	α_g	α_g (Calcd.)
He	4.35	0.15	2.13	...
H_2	6.47	2.27	7.8	8.3
N_2	14.7	10.5	17.6	17.8
Air	14.9	10.7	...	...
CO_2	28.8	24.6	26.6	28.2
SO_2	58.9	54.7	40.6	...
CH_3Cl	76.5	72.3	46.3	46.7

Expt. 50. Relative gain = 14.6.

Electronic Counting Experiments. Count rate data were plotted against discriminator bias and a smooth integral counting curve was drawn. Results obtained with a single counting channel, and not corrected for the small background count, are illustrated by Figure 2. Smoothed values of the count rates were read from the integral curves at constant bias intervals. The differences between successive rates are the count rate frequencies within the interval, and were taken as those corresponding to the mid-points of the intervals. The resulting frequency distribution, which is shown graphically in Figure 3 for the data of Figure 2, was treated by the usual statistical procedures (*23*) to obtain the mean pulse height and the standard deviation from the mean. The correction of the standard deviation for the effect of artificially grouping a continuous distribution into a discrete distribution turned out to be negligible (*5*), with the intervals chosen, compared to the probable error in the standard deviation.

Results obtained on three latex preparations are summarized in Table II. D is the mean particle diameter in microns; S/N is the ratio of mean signal to a noise level of 300 counts per minute; i_p is the normalized mean peak pulse photocurrent, in amperes; σ is the standard deviation from the mean; and K is a factor given by

$$K = 10^5\, i_p^{1/2}/D \qquad (1)$$

The value of i_p refers to multiplier anode current at the gain of unity and a standard source intensity (*W*, experiment 11). It was obtained from the discriminator voltage, amplifier gain, and the ratio of the air-scattering signal in the experiment to that in experiment 11. Thus, air served as the scattering standard in counting experiments.

Experiments 11 and 12 were conducted with a single channel and with no restorer circuit. An example of the other results with the two-channel apparatus of Figure 1 is given in Figure 4. In these, a factor, $(1 + 0.4\ N/S)^{-1}$, was applied to values of i_p to correct for the shift of average bias level produced by rectification of the random noise. The numerical factor was estimated to be 0.4 ± 0.1 by two different methods, taking into account the statistical distribution of the pulses in random noise and the frequency response characteristics of the instrument.

Typical curves of integral count rate for counts due to noise at several photomultiplier gains are shown in Figure 5. The zirconium arc source was used during these experiments, the

background light levels were typical, and the electrical delay was not used. Nonlinear scales are used in order to obtain approximately straight lines to facilitate extrapolation. The ordinate is logarithmic and the abscissa is linear in the square of the bias voltage.

An additional experiment was conducted on the 0.514-micron polystyrene to determine the magnitude of the error introduced by double counting. One channel contained a delay circuit and the other did not. Count rates at equal discriminator settings were found to be the same on the flat portions of the count rate curve. In the region of the mean pulse voltage, the circuit without delay gave count rates around 10% higher than the other. The excess was attributed to multiple counting of single pulses.

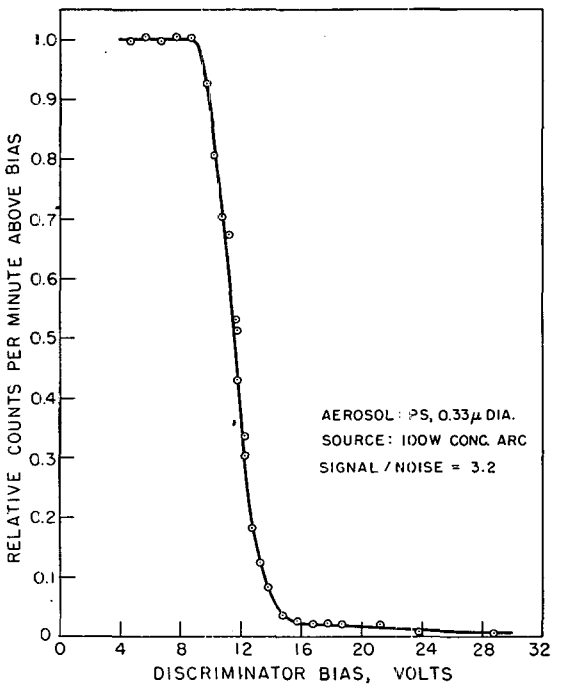


Figure 4. Integral count rate curve (experiment 54)

Photometric Measurements on Gases. The results of measurements of the light-scattering intensities from various gases at 25° C. and 752-mm. pressure are shown in Table III. The total photocurrent, i, was expressed as the sum of two parts, i_{sl}, due to stray light, which was considered constant, and i_g, due to scattering from the gas, proportional to the square of the molecular polarizability (*29*), α_g. Thus,

$$i = i_{sl} + i_g = i_{sl} + k\alpha_g^2 \qquad (2)$$

where k is proportional to the number of molecules per cubic centimeter, and is a complicated function of the intensity and spectral distribution of the source, various parameters of the optical system, the sensitivity and spectral response of the photocathode, and the gain in the photomultiplier. Corrections for the depolarization of the scattered light, which would be small, were not applied. From the data on helium and sulfur dioxide, i_{sl} and k were evaluated, yielding, respectively, 4.20×10^{-9} ampere and 3.32×10^{39} amperes per cc.[2] Values of i_g given in column 3 were calculated by means of the Lorentz equation (*2*) from the refractive indices of the gases for the sodium D lines (*20*), with the exception of methyl chloride, which was calculated from molar refraction obtained from atomic refractions (*1,6*). The molecular polarizabilities calculated from these results,

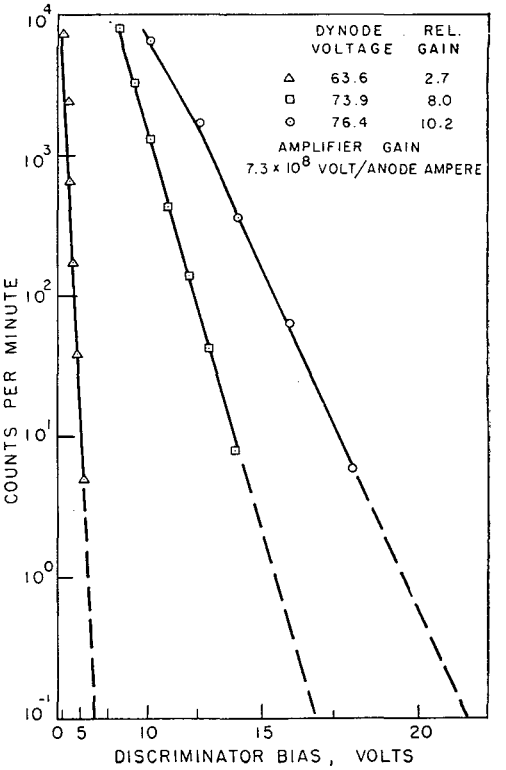


Figure 5. Count rate produced by noise

using helium and sulfur dioxide as standards, are given in column 5. Figure 6 is a logarithmic plot of i_g *vs.* α_g, which is a straight line with a slope of 1.99.

With nitrogen in the cell, the estimated noise on the recorder was about 0.3% of i. The drift rate amounted to 0.04 i per hour. The estimated precision in measuring i_g for nitrogen was 1%.

DISCUSSION

Monodispersity of Test Aerosols. Before and after the experiments with the hydrosols, the Tyndall spectra were observed in the usual manner (*28*), after dilution to reduce secondary scattering to a negligible value. The 1-micron particles gave five red bands, and 0.5-micron particles, two, and the 0.33-micron particles, one. The spectra were always clear, indicating that the preparations were uniform and stable by this criterion.

Given a monodisperse hydrosol, several variables must be controlled in order to produce a monodisperse aerosol by the spray-dry technique. Possible sources of heterodispersity are: coagulation of particles in the aerosol state; presence of dust in the solvent employed to dilute the hydrosol, or in the air streams; formation of aggregates by the evaporation of droplets containing more than one particle; formation of particles by evaporation of droplets containing only dissolved materials; deposition of dissolved substances upon a hydrosol particle by evaporation of solvent from the droplet; and formation of smaller particles by fracture of the hydrosol particles during atomization.

COAGULATION. The particulate concentration of the aerosol is so low under the conditions of these experiments (< 80 per cc.) that the coagulation rate (*30*) of the aerosol is several orders of magnitude less than required to prevent difficulties from this source. Coagulation of the spray droplets, before dilution and evaporation, would be expected to be relatively more important, particularly during atomization in the air jet. However, this can be taken into account by considering the final distribution of sizes of droplets in the spray, discussed herewith.

Contamination from Dust. Counting experiments were conducted to test possible contamination by particles in the filtered air line, and in the distilled water used to dilute the hydrosols. The filtered air count rate was negligible, typically on the order of 1 count per minute. When diluting water was spray-dried under the experimental conditions, the count rate was also low, and errors in the applied correction were negligible.

Formation of Aggregates by Evaporation. From what is known about the behavior of aerosol particles with respect to coagulation and filtration, it appears reasonable to assume that all of the hydrosol particles within a droplet will form an aggregate upon evaporation of the droplet. The probability of aggregation will then equal the probability that the droplets will contain two or more particles. This is a function of the droplet size of the spray. Such rough droplet size determinations as have been made on sprays from atomizers (*27*) lead one to expect that the mass median diameter of droplets produced in these experiments was between 1 and 5 microns, and that only a small fraction of the number of droplets were of a diameter greater than 6 microns. At a particulate concentration of the hydrosol of 10^8 per cc., the probability that a droplet 6 microns in diameter will contain one particle is around 10^{-2}. The probability that a doublet will be produced by evaporation of any 6-micron droplet is then 10^{-4} or 1% of the number of singlets, that of triplets is 0.01%, etc. Thus, the formation of aggregates by evaporation of the droplets was estimated to be negligible.

Formation of Particles from Droplets Containing Dissolved Materials Only. The distribution of particulate masses from this source can be calculated from the weight concentration of dissolved materials in the solution and the spray droplet size distribution. Some useful relations were obtained for computations of this sort.

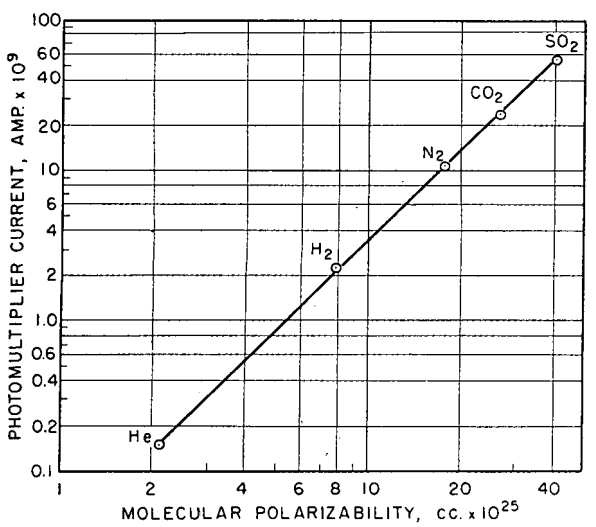


Figure 6. Light scattering from gases

Let f_l be the volume fraction of solids in the undiluted hydrosol; D_l the diameter of the latex particles (microns); n_l the number of latex particles per cc. in the original preparation; c_s the weight concentration in undiluted hydrosol of dissolved nonvolatile materials—e.g., stabilizer (grams per cc.); n_d the desired number of latex particles per cc. in the diluted hydrosol; F the dilution factor required; D_d the diameter of a spray droplet (microns); w_r the weight of the residue (grams) from evaporation of a spray droplet of diameter D_d; d_r the density of the residue (grams per cc.); and D_r the diameter (microns) of a residual sphere formed by the stabilizer. Then

$$F = n_l/n_d \tag{3}$$

where

$$n_l = 6 \times 10^{12} f_l/\pi D_l^3 = 1.91 \times 10^{12} f_l/D_l^3 \tag{4}$$

and n_d is determined from aggregation considerations, discussed previously. The weight of the residual particle (grams) can be calculated from the relation

$$w_r = 10^{-24}\, \pi^2 c_s n_d D_d^3 D_l^3/36 f_l \tag{5}$$

and its diameter in microns is

$$D_r = 10^{-4}(\pi c_s n_d/6 f_l d_r)^{1/3}\, D_d D_l \tag{6}$$

The relations are readily extended to characterize the distribution of particle sizes, when the distribution of droplets in the spray is known.

Taking $c_s = 0.005$ gram per cc., $n_d = 10^8$ per cc., $f_l = 0.1$, $d_r = 1$, $D_d = 6$ microns, and $D_l = 1$ micron, one obtains $D_r = 0.083$ micron, which is a rough estimate of the size of the largest residual particles expected in the case of the latex 1 micron in diameter, where the residual particles were the largest.

In a separate experiment, a hydrosol consisting of equal particulate concentrations of the particles 0.986, 0.514, and 0.333 micron in diameter, totaling around 10^8 per cc., was spray-dried, and count rates were obtained over the entire sensitivity range. The inflections corresponding to 1- and 0.5-micron particles were clearly resolved, but a large background occurred slightly below the level of the signals from the 0.3-micron particles. This may be attributed to the residual particles of stabilizer, entering mainly from the 1-micron hydrosol, or to fracture, discussed below. It was concluded that for any given preparation, residual particles are relatively unimportant, but when mixtures are taken, errors from this source may become significant.

Growth of Latex Particles by Deposition of Dissolved Materials. Since the volume of residue under the usual conditions turns out to be small compared to that of a particle, it can be shown that

$$\Delta r/D_l = 10^{-12}\, \pi c_s n_d D_d^3/36 f_l d_r \tag{7}$$

where Δr is the thickness of a uniform shell of residue, of density d_r, from a droplet of diameter D_d, obtained from the diluted hydrosol containing n_d particles per cc., and other terms are defined above. Under the typical conditions, $\Delta r/D = 10^{-4}$, assuming $d_r = 1$. For the case of mixed particles (see above), $\Delta r/D \leq 10^{-2}$, even if d_r is as low as 0.1. In this manner, it was concluded that size variations from deposition of dissolved materials were negligible.

Production of Smaller Particles by Fracture. In two experiments with the 1-micron polyvinyltoluene, not reported here, sharp breaks were observed in the count rate curves at the expected discriminator settings, but the count rates fell off significantly from the noise level to the breaks. Observations of the Tyndall spectra gave no indication of change of the hydrosols. This suggested that under certain conditions, not yet defined, some of the particles are fractured in the atomization process. The relatively large standard deviation in experiment 11-W-PVT was attributed to such fracture. Separate experiments indicated virtually complete fracture when sulfur sols 1 micron in diameter were spray-dried. In the case of the latexes, uncontrolled variables may affect the probability of fracture in the spray-dry technique. While this point requires further study, it was noted that the positions of the major breaks in the count rate curves agreed with these in other experiments, so the latexes are useful as calibrating materials in spite of this effect.

Scattering Intensity *vs.* Particle Size. Because the refractive indices of polystyrene and polyvinyltoluene are practically equal (*21*), the results of the experiments on the two may be compared directly. Values of K obtained with the zirconium concentrated arc source were not significantly higher than those obtained with the tungsten source, although the ratio of signal to noise was considerably greater. Following this unexpected result, normalized plots of the emission curves for the tungsten (*26*) and the concentrated arc (*4*) sources were made. Within the spectral region of interest, 3750 to 5750 A., determined by the product of the response of the multiplier tube and the relative emission from the source, the curves are practically superposable.

The probable error in determination of K was around 3%. Hence $K = 9.0 \times 10^{-5}$ within experimental error, which suggests the remarkably simple result that for many practical purposes the square law may be used throughout the range 0.33 to 1 micron. Such a response is also found (*24*, *28*) when $\alpha = \pi D/\lambda >> 1$, where the laws of geometric optics are applicable. For D = 1 micron, α is already greater than 7, so it may be expected that the same K will apply up to microscopically large drops. Whether or not the same constant applies is a point requiring further study. As more complete tabulations of the light-scattering functions for isotropic spheres become available (*3*, *10*, *11*, *18*, *19*), it may become practical to compute the response of the instrument to such particles as a function of refractive index and diameter. As such computations involve integrations over the spectral response region, and the solid angles of the illuminating and viewing systems, they are extremely laborious. It appears that the more expeditious procedure at the present time is to calibrate the instrument with uniform spherical aerosols by techniques like those described here.

Sensitivity of Counter to Small Particles. A current is set up in the photomultiplier by the background light flux, which is produced by scattering of the incident light by the gas within the total scattering volume, and by stray reflections and diffraction effects. In all the optical systems tested (*13*, *14*, and this research) background photocurrent was at least an order of magnitude greater than the dark current. Shot fluctuations in this photocurrent produce the random background signal referred to as noise, which is superposed upon the signals from particles, and sets a limit on the smallest signal that can be detected. With the final optical system, used in experiments 53 and higher, the signal-to-noise ratio was 3.3 for the particles 0.333 micron in diameter. Because of the rapid decrease of noise count rate with increasing discriminator voltage (Figure 5), setting the discriminator to a voltage 50% above the noise level of 300 counts per minute reduced the rate to about 1 count per minute. At this voltage, over 98% of the particles were counted.

With the tungsten source, the lower limit of size which can be counted is 0.3-micron diameter. This figure corresponds to practically complete counting of particles, with the noise contributing about 1 count per minute. The lower limit with the zirconium source will be very near 0.25-micron diameter. The tungsten source is preferred for most applications because of its greater stability. The magnitude of the concentrated arc source intensity fluctuations is quoted in the discussion of resolving power of the counter below. Such fluctuations could be tolerated only because of the extremely low stray light in the optical system. In cases where sensitivity and resolving power in the 0.3-micron region is of primary importance, the concentrated arc sources are to be preferred. The 300-watt unit could be used at an image-to-object ratio of unity, with some improvement in sensitivity over the 100-watt source as employed here.

The sensitivity to small signals can be approximately doubled by employing helium gas to dilute the aerosol. It was determined that this would reduce the background flux by a factor of 3.3, and decrease the noise level by a factor of 1.8. Additional improvement could be obtained with more intense light sources, and further decreases in the stray light. Reduction of the sensing volume would simultaneously decrease stray light, and raise the limits of the useful concentration range. Reduction of flow rate would reduce the required band width in the detecting system and decrease the noise level, but the maximum count rate would be decreased. Improvements of 100-fold in stray light, source intensity, or band width would increase the signal-to-noise ratio by approximately 10, and decrease the diameter of the smallest particle which can be detected by a factor which is estimated to be between 1.5 and 2.5. The factor in particle diameter is small relative to the improvement in signal-to-noise ratio, because of the rapid decrease of signal per particle below around 0.3-micron diameter.

Resolving Power of Counter. Employing the observed dependence of the signal upon the particle diameter, the measured standard deviations in the diameter were computed for the experiments with the concentrated arc source (Table IV).

Table IV. Standard Deviations in Particle Diameter

Diameter of Particle, μ	Standard Deviation, μ
0.986	0.080
0.514	0.036
0.333	0.018

The standard deviations may be regarded as a measure of the resolving power, or resolution, of the particle counter. Equal peaks in a distribution consisting of two sizes only should be clearly visible if the separation between the peaks is three times the standard deviation. With uniform particles, the uncertainty in determining the mean diameter will be less than the standard deviation by a factor depending somewhat upon the number of particles characterized.

If the pulses obtained from all the particles of a given size were exactly equal in amplitude, one might expect to make the resolving power as high as desired by refining the electronic discriminator circuits to the desired degree. However, the present results indicate that the attainable resolving power is determined by other factors, which may include variations in pulse length, coupled with limitations of the frequency response of the amplifier, superposition of random noise upon the signals, variations in illumination of the particles, variations of the aperture of the light-collecting system, and count rate errors.

Pulse Length Variations and Frequency Response Limitations. If the scattering signals were of equal peak amplitudes, but nonequal durations, the amplitudes of the electrical signals would be spread into a distribution which would be a function of the distribution of pulse durations and the frequency response of the electrical system. The over-all frequency response was determined primarily by the time constant of the photomultiplier output circuit, which was 0.2 msec. For 1-msec. pulses the resulting spreading was estimated to be around ±5% for durations varying by ±50%, and ±1% for a variation of ±10%.

Pulse durations were measured in the oscillographic experiments. The average deviation of the mean was 10%, which was the estimated precision of the measurements. This indicates that the flow system possesses the desired characteristic that all particles are illuminated for periods which are equal within the precision of the measurements. Thus, variations in pulse length and limitations of frequency response were relatively unimportant in this work.

Effect of Random Noise. With signals of exactly equal amplitudes, there will be a spreading of the measured pulse amplitude distribution produced by the random noise. The noise level that is pertinent here is that corresponding to the flux prevailing when the particle is within the sensing volume. The noise is proportional to the square root of the background flux (*8*). For very small particles the increase of noise produced by scattering from the particle is negligible. For large particles which produce a photocurrent much greater than the background, the noise level increases approximately as the square root of the signal. Then the signal-to-noise ratio will be proportional to the square root of the signal, or to the diameter of the particle. From these considerations, it was concluded that the percentage spread of the pulse amplitudes produced by noise should vary inversely as the first power of the diameter for large particles (>0.5 micron), and more rapidly for smaller ones. As the deviations were essentially constant for particles 1, 0.5,

and 0.3 micron in diameter they cannot be attributed solely to random noise. This was verified by some approximate computations, employing the count rate curves of Figure 5, which showed that noise could account for only a fraction of the deviation even in experiments 59 and 60, where S/N values were lowest.

VARIATIONS IN ILLUMINATION. Here one must consider the stability of the light source and the uniformity of illumination over the sensing volume. The tungsten source was stable within around 1% during the counting experiments. The zirconium arc sources were stable to within 1 or 2% in certain experiments; in others, variations of 10% were sometimes observed. The maximum standard deviation in any one experiment was 5%. From earlier measurements (*13*) it was estimated that the optical field was uniform to around ±2% with a tungsten source, and ±10% with the zirconium source. From this it appears that variations in illumination cannot entirely account for the observed deviations.

NONUNIFORMITY OF APERTURE OF COLLECTING SYSTEM. This factor should be investigated in detail if higher resolving power is desired. It can be argued that because the possible range of positions of the aerosol particles is small compared to the focal length of the lenses, little spread of signals is to be expected from this source. However, in considering the limited size of *S*9, and the practical limitation of lenses, it appears that most of the standard deviation might be due to this factor. This would be consistent with the observed constancy of the deviation. Experiments should be conducted with *S*9 of larger dimensions.

COUNT RATE ERRORS. When count rates are measured with a single channel counter, errors are introduced by variations of the aerosol concentration and the sampling rate during the period of a complete experiment, which was around 1 hour. This is illustrated by the scatter of the points in Figure 2 between 1 and 10 volts. Portions of the standard deviations in experiments 11 and 12 can be attributed to these variations. In other experiments, with the two channels, the counting procedure automatically compensated for the concentration and fluctuations in sampling rate, so errors of this type were eliminated (see Figure 4). While statistical errors did not play a significant role here, in the general case, a multichannel counting system would be superior.

Sensitivity and Stability of Photometer. The precision of the normalized mean pulse currents depends partly upon the uncertainties in the measurements of the photocurrent due to light scattering from air. The air scattering was obtained from the difference between the total photocurrents with air and with helium in the total scattering volume of the cell. No corrections were applied for the variations in barometric pressure and room temperature. These together could introduce an uncertainty of the order of 2%. The error estimated from all causes, for experiments employing the tungsten source, was 3 to 4%. Larger estimates of 7% for experiment 53 and 5% for experiment 54 reflect the additional contribution of intensity fluctuations of the zirconium source.

For photometric measurements, the advantage of the larger scattering photocurrents obtained with the zirconium source is offset by the greater instability, which makes it less suitable than the tungsten source.

The probable error of the measurements of the total flux with gases in the cell, listed in column 2 of Table III, was at most 2%. The disagreement between columns 4 and 5 is significantly larger than this figure for two cases, hydrogen and carbon dioxide. For hydrogen this probably arises because the gas scattering photocurrent is a small fraction of the total. In the case of carbon dioxide, depolarization corrections (not applied) and impurities may be contributing factors. In Figure 6, the straight line with slope 1.99 is in excellent agreement with the value 2.00 predicted by the Rayleigh theory.

For comparison with previous photometers, it is convenient to state the sensitivity in terms of an aerosol 0.3 micron in diameter with a refractive index around 1.5. It was calculated (extrapolating slightly the square-law response down to 0.30 micron diameter) that 220 particles per cc. of a test aerosol 0.3 micron in diameter (mass concentration of 3×10^{-9} gram per liter) would produce a scattering signal equal to that from pure air. With the tungsten source, the smallest detectable concentration is 1% of this or 3×10^{-11} gram per liter. This is a 3 times greater sensitivity than that achieved earlier (*16*). With the aerosol confined within the central stream, the corresponding limit is 7×10^{-11} gram per liter.

ACKNOWLEDGMENT

The work was supported by the Office of Naval Research. The authors are happy to acknowledge the assistance of Moshe D. Bitron, and Wm. I. Higuchi, T. J. Flautt, and John Applequist in obtaining some of the experimental results.

LITERATURE CITED

(1) Bauer, N., and Fajans, K., "Physical Methods of Organic Chemistry," A. Weissberger, ed., Chap. XX, Interscience, New York, 1949.
(2) Böttcher, C. J. F., "Theory of Electric Polarisation," Elsevier New York, 1952.
(3) Boll, R. H., Gumprecht, R. O., and Sliepcevich, C. M., *J. Opt Soc. Amer.*, **44**, 18 (1954).
(4) Buckingham, W. D., and Deibert, C. R., *J. Soc. Motion Pictur Engrs.*, **47**, 376 (1946).
(5) Cramer, H., "Mathematical Methods of Statistics," pp. 359–63 Princeton University Press, Princeton, 1951.
(6) Eisenlohr, F., *Z. phys. Chem.*, **75**, 585 (1910).
(7) Elmore, W. C., and Sands, M., "Electronics," McGraw-Hill New York, 1949.
(8) Engstrom, R. W., *J. Opt. Soc. Amer.*, **37**, 420 (1947).
(9) Ferry, R. M., Farr, L. E., Jr., and Hartman, M. G., *Chem. Revs.* **44**, 389 (1949).
(10) Goldberg, B., and Penndorf, R., to be published in Geophysical Research Directorate, Air Force, Cambridge Research Center Mass.
(11) Gucker, F. T., Jr., and Cohn, S. H., *J. Colloid Sci.*, **8**, 55 (1953).
(12) Gucker, F. T., Jr., and O'Konski, C. T., *Chem. Revs.*, **44**, 37 (1949).
(13) Gucker, F. T., Jr.,and O'Konski, C. T., *J. Colloid Sci.*, **4**, 54 (1949).
(14) Gucker, F. T., Jr., O'Konski, C. T., Pickard, H. B., and Pitts J. N., Jr., *J. Am. Chem. Soc.*, **69**, 2422 (1947).
(15) Gucker, F. T., Jr., Pickard, H. B., and O'Konski, C. T., "Handbook of Aerosols," Chap. 10, Atomic Energy Commission Washington, D. C., 1950.
(16) Gucker, F. T., Jr., Pickard, H. B., and O'Konski, C. T., *J. Am. Chem. Soc.*, **69**, 429 (1947).
(17) Gucker, F. T., Jr., and Rose, D. G., *J. Appl. Phys.*, **25**, Suppl. 3 (1954).
(18) Gumprecht, R. O., and Sliepcevich, C. M., *J. Phys. Chem.*, **57**, 90 (1953).
(19) Gumprecht, R. O., Sung, N., Chin, J. H., and Sliepcevich, C. M., *J. Opt. Soc. Amer.*, **42**, 226 (1952).
(20) "Handbook of Chemistry and Physics," 29th ed., p. 2188, Chemical Rubber Publishing Co., Cleveland, Ohio, 1945.
(21) Heller, W., Epel, J. N., and Tabibian, R. M., *J. Chem. Phys.*, **22**, 1777 (1954).
(22) Higinbotham, W. A., *Rev. Sci. Instr.*, **22**, 429–31 (1951).
(23) Hoel, P. G., "Introduction to Mathematical Statistics," Chap. II, Wiley, New York, 1947.
(24) Hulst, H. C. van de, "Optics of Spherical Particles," pp. 28, 62, N. V. Drukkerij, J. F. Duwaer & Zn., Amsterdam, 1946.
(25) Radio Corp. of America, Harrison, N. J., "RCA Tube Handbook 3," Vols. 1–2, Sheet **92CL-6454 R2** (Oct. 26, 1949).
(26) Reich, H. J., "Theory and Applications of Electron Tubes," p. 539, McGraw-Hill, New York, 1944.
(27) Rosebury, T., "Experimental Air-Borne Infection," pp. 80–2, 87–9, 131–4, Williams & Wilkins, Baltimore, 1947.
(28) Sinclair, D., "Handbook on Aerosols," Atomic Energy Commission, Washington, D. C., Chap. 7, 8, 1950.
(29) Stuart, H. A., "Molekulstruktur," J. Springer, Berlin, 1934.
(30) Whytlaw-Gray, R., and Patterson, H. S., "Smoke," Edward Arnold & Co., London, 1932.

RECEIVED for review September 3, 1954. Accepted February 28, 1955.

Microdetermination of Sulfuric Acid Aerosol

EARL R. GERHARD[1] and H. F. JOHNSTONE

University of Illinois, Urbana, Ill.

An analytical technique for the microdetermination of sulfuric acid aerosol was developed in which the aerosol particles were collected by a high velocity impactor on a film impregnated with thymol blue. The color change from yellow to red, resulting from the action of the sulfuric acid drops, was measured by a photoelectric colorimeter attached to a low power microscope. The film was calibrated by impaction of known quantities of sulfuric acid aerosol. The method was used to determine from 0.01 to 0.2 γ of sulfuric acid. It was reliable when the quantity of acid collected was controlled to give essentially uniform acid traces. The method detects the presence of any acid aerosol, but is not sensitive to acidic gases. Alkaline gases which tend to neutralize the droplets affect the accuracy of the results.

CONSIDERABLE difficulty has been experienced in air pollution work both in the field and in the laboratory because of the small quantities of materials available for analysis. The analytical technique described here was developed while studying the rate of formation of sulfuric acid aerosol from the photochemical oxidation of sulfur dioxide in the presence of water vapor. It was desired to sample the sulfuric acid aerosol formed in an 8-liter reaction chamber when 1 to 30 p.p.m. of sulfur dioxide was used. At 1 p.p.m. of sulfur dioxide in air the concentration of sulfur dioxide is 2.85 γ per liter. Even if all the sulfur dioxide is converted to sulfuric acid, 1 liter would contain only 4.37 γ of sulfuric acid.

Ordinary methods of acid analysis, such as acid-base or amperometric titrations, could not be used to determine less than 100 γ of sulfuric acid. At first, an attempt was made to count and measure, under the microscope, the particles collected on an impactor slide, but this was impossible because of the presence of many small particles about 0.5 micron in diameter, even in filtered air. By dissolving the acid collected on the impactor slides in small quantities of water and measuring the electrical conductivity, quantities as low as 5 γ could be determined. The method possibly could be extended to determine as little as 1 γ of acid.

The work led to the development of a method using indicator films. Strips of film were impregnated with solutions of methyl red, neutral red, methyl orange, bromocresol green, and thymol blue. Best results were obtained with the bromocresol green and thymol blue. Further tests were made with these two indicators to determine the optimum concentration and pH of the indicator solution and the time of immersion of the film in the solution. The selection was based on the maximum light transmittance difference between the acid and base colors.

The aerosol was sampled by jet impaction, a method originally developed by May (*1*). The particular jets used in this work and their calibration are described by Ranz and Wong (*2*). With a round jet 0.353 mm. in diameter and at sonic velocity through the throat, it was possible to obtain essentially 100% removal of 0.20-micron droplets and larger, and 50% removal of 0.11-micron droplets.

PREPARATION OF FILMS

Eastman positive, fine-grained, 35-mm. photographic film was immersed in a hypo solution to remove the silver salts, then washed with distilled water, and dried. Two grams of thymol blue was dissolved in 90 ml. of 0.05*N* sodium hydroxide in an agate mortar, filtered through No. 1 Whatman filter paper, and diluted with distilled water to 1 liter to give approximately 0.2% by weight. The solution was adjusted with 0.1*N* sulfuric acid to a pH of 3.3. Two-foot strips of the film were immersed in the solution and stirred for 2 minutes. The film was dipped in distilled water, and rinsed once on each side with a stream of distilled water. It was then dried and stored in filtered air in a box.

The same technique was used to prepare the bromocresol green film. For this film, the solution concentration was 0.1% by weight and it was adjusted to pH 4.4.

PHOTOELECTRIC COLOR MEASUREMENTS

A section of the indicator film was attached by Scotch tape to a small microscope slide. The sulfuric acid aerosol particles were impacted on the film by the round jets described previously. Approximately circular traces between 0.35 and 1.0 mm. in diameter, depending on the jet size and quantity of acid impacted, were obtained. The difference in light transmittance between the acid colored trace and an adjacent base colored part of the film was then determined. For this purpose, a photosensitive element from a Cenco photelometer which had an inside diameter of 2.5 cm. was used. In order to obtain sufficient sensitivity and significant differences in light transmittance, it was necessary to

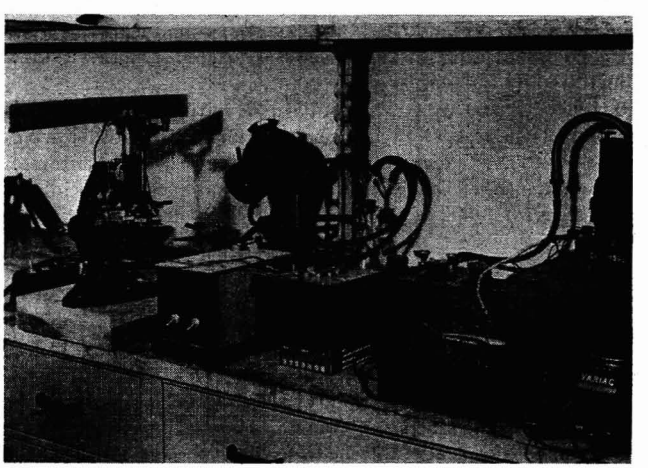


Figure 1. Apparatus used for microdetermination of sulfuric acid aerosol

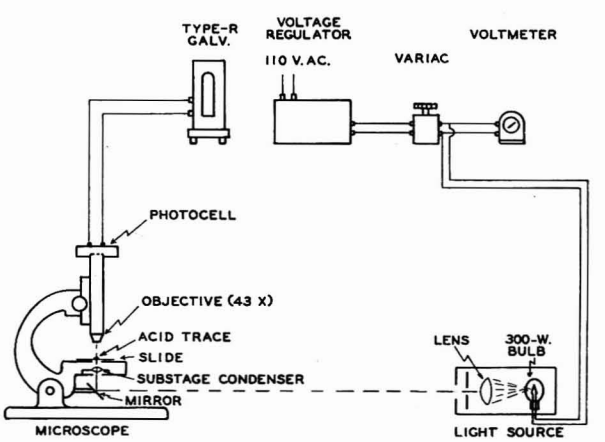


Figure 2. Optical arrangement of apparatus

[1] Present address, Department of Chemical Engineering, University of Louisville, Louisville, Ky.

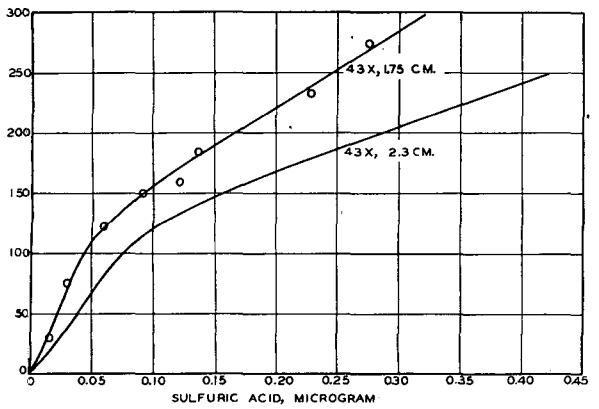


Figure 3. Calibration of indicator film

magnify the trace or dot size. This was accomplished by use of a 43× objective on a Bausch & Lomb microscope in which the eyepiece was replaced by the photocell.

A photograph of the apparatus used is shown in Figure 1. The optical arrangement is shown in Figure 2.

A light source of constant intensity was adjusted to give a narrow beam of parallel light by means of a lens and diaphragm. The distance from the light source to the microscope was set at 45 cm. to permit adjustment of the size of the beam to the proper diameter. The photosensitive element was connected to a Leeds and Northrup Type R galvanometer which had a sensitivity of 0.005 μa. per mm. at 1 meter.

In order to increase the transmittance difference between the acid and the base color, Wratten filters 58B and 15G were placed in the light beam between the mirror and substage of the microscope.

The acid trace or dot could be observed and focused by placing a frosted disk on top of the microscope barrel. For maximum transmittance differences, it was necessary to use a light beam of approximately the same size as the acid dot. For this purpose a series of brass disks with circular holes was used. One of these was placed on top of the frosted disk. For each reading, a disk which just enclosed the magnified projection of the acid dot was selected, and the substage diaphragm was adjusted to give a beam of light of the same diameter as the disk.

Before readings were taken, the mirror was adjusted to give a uniform and centered field of illumination as observed on the frosted disk; the galvanometer was set at zero with no light on the photocell. The substage condenser was adjusted to focus the substage diaphragm on the frosted disk and the diaphragm was closed to give the proper size light beam. For each film, the light intensity was set to give a galvanometer reading of 50.0 cm. for the base color of the thymol blue film. Transmittance readings were obtained between 20.0 and 45.0 cm. for the acid dots. This procedure gave reproducible transmittance readings.

CALIBRATION OF FILMS

The thymol blue indicator films were calibrated by impaction of a known quantity of sulfuric acid aerosol on the films. A condensation aerosol generator similar to that used by Sinclair and LaMer (*3*) for aerosol research was used to produce homogeneous sulfuric acid aerosols with particles about 0.6 micron in diameter.

The aerosol stream from the generator was diluted with filtered air and passed through two mixing chambers. All of the particles in the stream were then collected in the impactor cup and analyzed for sulfuric acid. The volume of gas passing the impactor was measured. During each run, 1- to 30-ml. samples of the air containing the aerosol droplets were removed from the mixing chamber and injected into a second filtered air stream, where the acid particles were impacted on the indicator films through a round jet. From the known concentration of the aerosol and the volume of the sample, the amount of acid deposited on the film was calculated.

In this way, acid traces containing 0.015 to 0.3 γ of sulfuric acid were obtained. The light transmittance differences of the acid traces, 0.35 to 0.41 mm. in diameter, were measured with the 43× objective and 1.75-cm. disk as described above. The calibration curve for these results is shown in Figure 3. Readings on larger dots having a uniform distribution of acid showed that in the range 0.41 to 0.535 mm. the transmittance difference depended only on the amount of acid collected per unit area. Thus, a uniform trace 0.535 mm. in diameter gave the same transmittance difference with a 2.3-cm. beam of light as with a 1.75-cm. beam, but contained more acid in proportion to the larger area. Based on these results, a calibration curve for 43× and 2.3 cm. was calculated from the experimental curve for 43× and 1.75 cm. This is also shown in Figure 3.

For example, assume that we have a dot 0.535 mm. in diameter which contains a uniform acid color over its entire surface. When this dot is read with the 43× objective lens and a 2.3-cm. beam of light, a difference of 250 mm. is obtained on the galvanometer. If now the size of the light beam is reduced to 1.75-cm. diameter, and the voltage to the light source readjusted to give a reading of 500 for the base color, the dot will again give a difference of 250 mm. on the galvanometer. The calibration obtained for the 1.75-cm. size gives a value of 0.24 γ of acid. Hence, when the 2.3-cm. beam of light is used, a reading of 250 would correspond to a total acid content of $(0.24)(2.3/1.75)^2 = 0.41$ mg.

This procedure was substantiated when experimental dots gave approximately the same total amount of sulfuric acid when measured by either of the two calibration curves.

APPLICATIONS AND LIMITATIONS OF INDICATOR FILM ANALYSIS

Use of these thymol blue indicator films permitted determination of 0.10 to 0.50 γ of sulfuric acid aerosol and detection of quantities as small as 0.01 γ. This is equivalent to 2×10^{-3} p.p.m. by volume of sulfuric acid vapor from a 1-liter sample. For many test runs an analysis could be made by removing less than 250 ml. of aerosol from the reaction chamber.

To ascertain the effect of filtered air on the film, 25 liters of air–sulfur dioxide mixtures containing up to 300 p.p.m. of sulfur dioxide, with and without sodium chloride nuclei, and gases such as nitrogen dioxide and hydrogen chloride, were drawn through the impactor at sonic velocity. No visible trace or color was observed under these conditions. The presence of other acidic or basic impurities dissolved in the aerosol of course modifies the color change caused by sulfuric acid alone. Furthermore, soluble salts might crystallize in the trace on the film with a decrease in light transmittance caused by the solid particles. Test runs in which a trace of ammonia gas was added to the air showed a marked decrease in the color of the aerosol trace and the presence of solid white crystalline particles on the film.

In general, the light transmittance readings of the films were made within 10 minutes after the sample was collected, and often within 2 minutes. Readings on several dense acid traces showed no significant change in transmittance for several days when the film was stored in a closed container in the dark. However, fading occurred after storage of several weeks. Before use, the films were perfectly stable and gave reproducible transmittance readings after storage for months.

The most serious difficulty was the tendency of the acid to splash or spread into irregular shapes, especially when large quantities were collected by impaction near sonic velocity. Occasionally, the high velocity of the jet blew the acid into streamers several millimeters from the central dot. When possible, these runs were repeated and smaller quantities of acid were collected. Traverses of traces having irregular shapes or slight spreading usually gave satisfactory results.

ACKNOWLEDGMENT

This work was a part of the program in the University of Illinois Engineering Experiment Station on a study of stack gases. It was supported in part by the Smoke and Fumes Committee of the American Petroleum Institute as Contract SF-9.

LITERATURE CITED

(1) May, K. R., *J. Sci. Instr.*, **22**, 187 (1945).
(2) Ranz, W. E., and Wong, J. B., *Ind. Eng. Chem.*, **44**, 1371 (1952).
(3) Sinclair, D., and LaMer, V. K., *Chem. Revs.*, **44**, 245 (1949).

RECEIVED for review September 3, 1954. Accepted December 21, 1954.

Gas Chamber Microapparatus in Identification of Air-Borne Pollutants

J. L. MONKMAN

Occupational Health Laboratory, Department of National Health and Welfare, Ottawa, Canada

In the identification of air pollutants, samples on microslides, glass wool, or other holders are exposed to reagents in the vapor phase. The reactions are carried out in gas chambers of borosilicate glass constructed from the two sections of a standard-taper joint. By breaking the chamber at the joint the sample may be inserted and the joint closed, after which the sample may be exposed to a gaseous reagent or to several in succession. During the vapor treatment, microslide samples may be withdrawn at intervals for microscopic examination and photomicrographing. The composition of the effluent gas may be investigated by the use of paper disks sensitized for gases such as arsine, or by infrared investigation of organic material caught on silica gel contained in absorbers downstream from the reaction chamber.

IN AIR pollution investigations, a variety of sampling devices have been developed to collect samples of the air-borne pollutants. Depending upon the sampling apparatus, the collecting medium, and the air being sampled, the "particulates" as collected may or may not approximate the original physical state and distribution of the particles in air. Because samples taken on microslides, membrane filters, and electron microscope films are fairly representative of the original distribution, if not the original physical state, and methods employing microscopy are essentially nondestructive, it was decided to investigate air samples taken on the types of sample holders mentioned. It was thought it might be possible to adapt the long established techniques of microchemistry on a microslide to air samples. Both light and electron microscopes could be used to make visual records of the pollutants through the medium of photographs.

For the identification of air pollutants under the microscope various techniques have been used, including chemical micrurgy, petrography, and x-ray diffraction (*1-3, 5, 7, 10*). In the identification of air-borne particulates it may be of interest to identify individual particles as originally deposited on the slide. Under favorable conditions an expert in petrography can identify and classify particles on a microslide without disturbing the sample. More often it may be necessary to characterize the particles by determining refractive indices in an immersion oil, in which case the original particle distribution may be altered. The usefulness of such physical methods is decreased as the particle size decreases, and is affected by other factors such as the birefringence of the material. Confirmatory chemical tests may be applied to individual particles by micromanipulation, but it would be too tedious to handle an entire microscope field in this way unless the material of interest was of infrequent occurrence in the samples.

It was thought that if the particles on the microslide could be exposed to suitable reagents in the gaseous state it might be possible to induce changes involving color, crystal structure, size, etc., without changing the location of the particle on the slide. Observation of the particles before and after the treatment with the gaseous reagent would serve as a basis of identification without affecting the particle frequency distribution. Photographic records could be made as reactions proceeded.

Microchemical reactions in which the unknown is acted upon by a gaseous reagent are not new. However, most of the work described in the literature was done on a closed or semiclosed system, so that free gas flow was not obtained. The sample was usually destroyed by solution in acid and the identification based on a suitable color test, etc., taking place at a point separated in location from the test solution. Gitzen has used a gassing technique in conjunction with the electron microscope to differentiate silica from alumina in furnace fumes (*4*). The method is not specific, however, and identification would not have been possible if the samples had not consisted of only silica and alumina.

A gassing technique has been used with submicron particulates by Leroux in a study carried out at the French National Research Council (*6*). Hydrochloric acid vapor was used to differentiate pneumoconiosis-producing silicates and quartz from other associated submicron particles. The electron microscope was used to follow the course of the reaction.

It was considered that morphological changes induced at the individual particle site might be an aid to identification. Because reaction velocity is likely to be more rapid in a stream of continuously flowing gaseous reagent, it was decided to make small gas chambers, within which the sample, on the appropriate holder, could be exposed to a gaseous reagent under conditions of continuous flow. However, morphological changes may not always be observable. In addition, samples containing arsenic, carbonates, sulfides, and sulfites may be expected with suitable treatment to liberate gaseous reaction products. For these two reasons, it was decided to make provision for testing the gas stream after it had passed the sample. Gaseous reaction products are arsine, carbon dioxide, sulfur dioxide, and hydrogen sulfide. Certain air samples might be expected to contain hydrocarbon residues from the combustion of coal, oil, and gasoline, which might be driven out of the sample in treatment, and should be looked for in the effluent gas stream. In microscopic observation of a single sample of air-borne material, subjected to treatment with gaseous reagents, there are four possibilities:

1. The particles may show obvious change reflected in the composition of the effluent gas stream.
2. The particles may show obvious change, not reflected in the composition of the effluent gas.
3. The particles may not show observable change but there may be a change in the effluent.
4. The particles may appear unaffected and the effluent may also indicate no change.

If the conditions of (1) apply, the maximum amount of information may be expected from the sample.

APPARATUS

As a simple gas chamber both sections of a ₹ 40/35 borosilicate glass joint have been used, as well as larger sizes. The free ends are drawn down and fitted with borosilicate glass stopcocks as in Figure 1. Two-way stopcocks make it convenient to change from one gaseous reagent to another, without having to remove the chamber from the train. It is also desirable to fit the free ends of the stopcocks with ball and socket joints, for ease in making connections, and to avoid the use of rubber. The purpose of the stopcocks is not to control gas flow, but to provide for the possibility of sealing the system at will and withdrawing the chamber from the gassing train, or to remove from one gassing train and insert in another.

Chambers made with ₹ 40/35 joints are ideal for microslides of the standard 1 × 1.5, 1 × 2, or 1 × 3 inch sizes. The chamber is held in a clamp in a horizontal position. To insert a slide in the apparatus it is necessary only to break the connection at the joint and insert the slide, the long edges of which rest on the walls of the glass tube at a vertical distance of 1 cm. above the lowermost portion of the gas chamber. The two halves of the joint are then connected and held together with springs. For larger sample holders, such as millipore filters, Whatman filter thimbles, and glass fiber filters, chambers are made from larger joints such as ₹ 45/50 and ₹ 55/50.

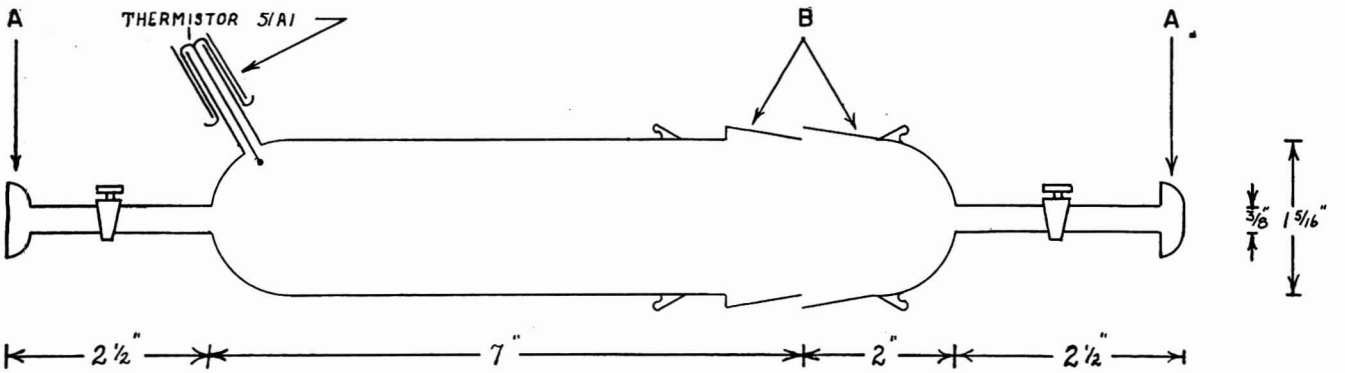


Figure 1. Gassing chamber with thermistor

A. S 10/7 *B.* ₹ 40/35

These chambers have been found rugged, corrosion-resistant, easy to clean, and leakproof in use. They are unaffected by ordinary gaseous reagents, except hydrofluoric acid. They are compact and portable, and may be used to take grab samples of air. Because it is relatively inexpensive to prepare a considerable number of such gas chambers, they are suitable for mass production of results. Although originally designed for use with microslides, they have been found equally useful for macro samples, which can be inserted in the apparatus in a suitable glass holder or on a microslide.

The basic design has been modified in various ways for special purposes.

Figure 1 shows a thermistor sealed into the wall of the chamber, which, when connected to an external Wheatstone bridge, can be used to measure the temperature inside the chamber. Another type (not shown) has two platinum rods sealed through the chamber wall 2.75 inches apart, upon which an electrical conducting microslide may be placed, which can then be heated by current supplied to the exterior ends of the platinum rods by a Variac.

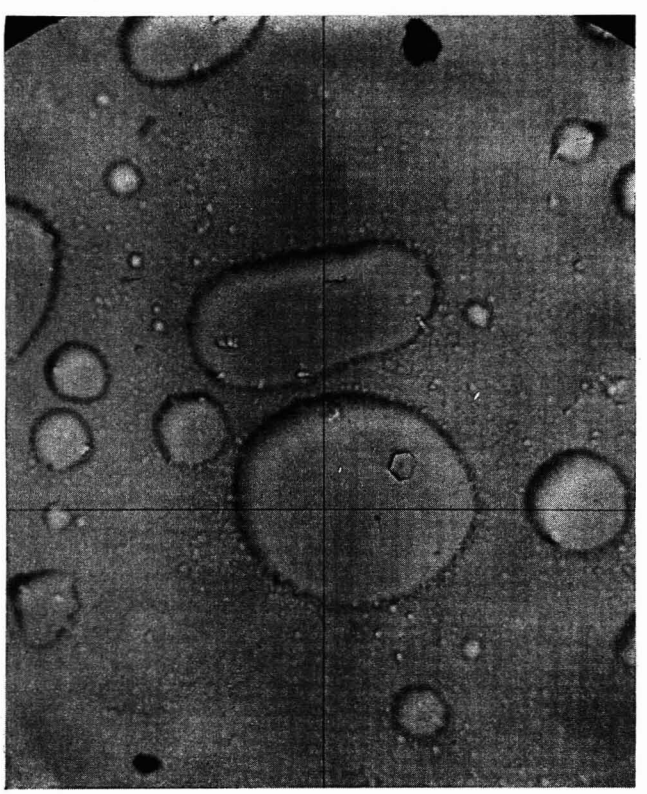


Figure 2. Calcium chloride crystal in droplet

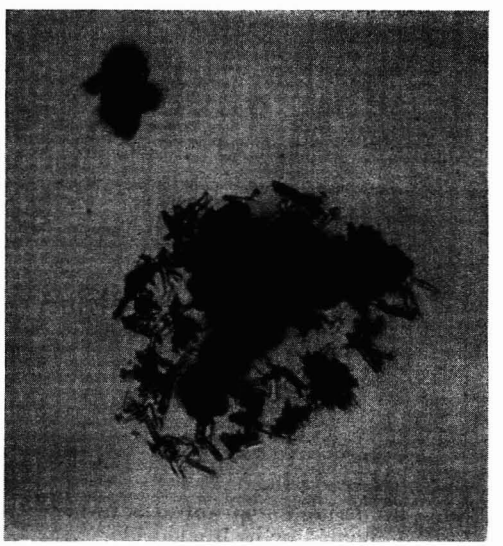


Figure 3. Gypsum crystals around air-borne calcite particle

Another heating chamber (not shown) contains a quartz frame, attached to one of the joint members, upon which is wound 18 inches of No. 28 platinum wire. The leads of the platinum heater are sealed through the chamber wall and current is obtained from a Variac. The microslide of glass, or quartz, is placed upon the platinum-quartz heater and introduced into the other section of the joint and the chamber is closed. Temperatures of 500° C. are easily reached with this heater and some care is necessary in its use, as ordinary glass microslides may be softened and distorted at high temperatures. By the addition of standard-taper 24/40 joints crosswise to the basic chamber (not shown), it is possible to expose the slide to the vapor of a liquid reagent refluxing from the condenser above to the boiling flask below. It is desirable to have the specimen slide on a heating element, which can be adjusted to present rapid condensation and flooding of the reagent vapor on the sample. When applicable (arsenic oxide), the gas chamber, with heating element, may be used for sublimation of a sample from one slide to another, or for fusion reactions with 8-quinolinol (*9*). The sealed-in thermistor elements may be used to measure flow rates instead of temperature, if desired.

REAGENTS

The following reagents were investigated: acetic acid, hydrochloric acid, nitric acid, oxygen, nitrogen, hydrogen, water vapor, alcohol, ammonia, and acetylacetone. Other liquid and gaseous reagents suggest themselves. Nitrogen and hydrogen may be used to provide inert atmospheres, and oxygen to provide an oxidizing, and hydrogen a reducing atmosphere. Acids such as

nitric and hydrochloric are best used by bubbling a carrier gas through an aqueous 10 or 50% aqueous solution of the acid contained in a sintered-glass bubbler.

METHOD

The sample, on a microslide or other holder, was placed in the gas chamber and all connections were made. The gaseous reagent of choice was then passed through the chamber at a rate determined by experience. As gassing of a sample proceeded, the slide was withdrawn from time to time for a microscopic examination. The slide was traversed with the mechanical stage in arbitrary fashion in the two horizontal dimensions in the search for changes from the original pre-exposure state. For record purposes, photomicrographs were taken when changes were observed, usually with a 10 × eyepiece and 10 × objective.

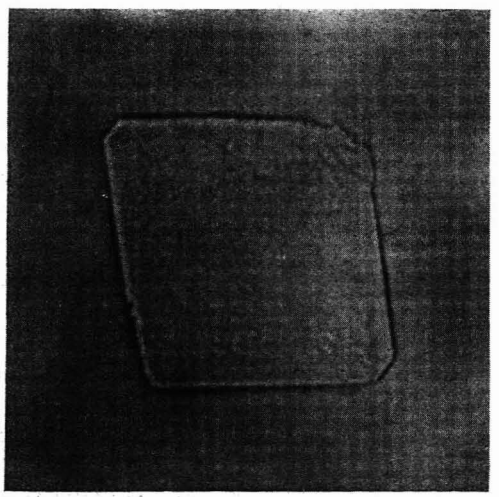


Figure 4. Reaction of sulfuric fume with benzidine hydrochloride

The effluent gas stream was tested by the use of sensitized test papers or silica gel adsorbent downstream from the chamber. Arsine and sulfur dioxide, for example, were caught in a special confined spot test apparatus developed in this laboratory. Organic compounds were adsorbed on silica gel and characterized by their adsorption in the ultraviolet in pure iso-octane (2,2,4-trimethylpentane), or by infrared absorption.

To provide specificity, or to localize a reaction product at the particle site, sensitized slides were also used. Transparent dry films of polyvinyl alcohol, or wet films of glycerol, containing reagents such as benzidine hydrochloride, sulfuric acid, potassium ferrocyanide, and 8-quinolinol, were used on a supporting microslide. For the sake of simplicity, rates of gas flow were arbitrarily set at 1000 ml. per minute.

RESULTS

Calcite. Gaseous reactions with samples of pure calcite were very rapid (several minutes) with acid vapors such as hydrochloric acid. The calcite particles are converted to droplets of calcium chloride solution, in which after some time hexagonal and rhombic crystals appear (Figure 2). With acetic acid vapor calcium acetate is formed, which may occur in very large rectangular crystals. Calcite particles are easily identified on a slide sensitized with sulfuric acid and gassed with water vapor or hydrochloric acid, by the characteristic ring of gypsum crystals which form around individual particles (Figure 3). Calcite particles coated with acrylic resin (Krylon) and exposed to hydrochloric vapor show bubbles of carbon dioxide evolving slowly at the site of the particle. Calcium chloride droplets, formed from calcite, may be exposed to the refluxing vapor of acetylacetone; this converts the chloride to the calcium chelate, which occurs in highly birefringent characteristic crystals.

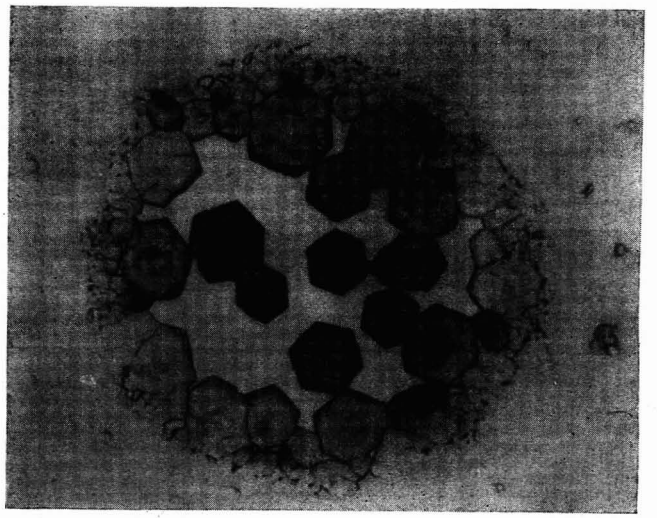


Figure 5. Crystals of cadmium chloride from cadmium oxide

Iron Compounds. Ferric oxide forms droplets with hydrochloric and nitric acids. Characteristic crystal structures are formed in the droplets later. With acetic acid, rectangular crystal forms are seen. The most useful test makes use of a sensitized slide containing ferrocyanide. Ferric oxide particles on such a slide when exposed to hydrochloric acid become surrounded by a blue halo, due to reaction with the iron. The reaction is very rapid, and is specific for ferric iron.

Arsenic. Ordinarily, except at certain specific locations such as

Figure 6. Reaction of zinc dust with acetylacetone

smelters, the amount of arsenic in air samples is insufficient to identify in individual particulate form. As it had been reported that arsenic in very small amounts could be identified by the characteristic form under the electron microscope (*8*), small measured amounts of arsenic, from 1 to 10 γ, were placed on slides and investigated under the microscope. It was found that characteristic hexagonal crystals could be located on the slides down to samples as small as 3 γ; below this amount the arsenic could not be identified by its form. It was concluded that it was better to identify arsenic by tests based on arsine evolution.

Test paper disks sensitized with mercuric bromide were placed downstream from the gas chamber. Beside the sample on a microslide was placed a pellet of arsenic-free 4-mesh zinc, a drop of 40% stannous chloride solution in concentrated hydrochloric acid was applied to the pellet of zinc, the slide was inserted in the chamber, and the system was closed. Suction was now applied from a water pump. By tilting the chamber slightly from the horizontal, the zinc and hydrochloric acid were made to slide into contact with the sample.

Using a spot test assembly having a cross-sectional diameter of 3.0 mm. it was possible to get visually positive stains for measured amounts of arsenic as low as 0.1 γ as As. This sensitivity can be improved by the use of a densitometer. Arsenic was found in most urban air samples tested.

Sulfates. Air-borne fumes of sulfuric acid have been caught on films containing barium chloride or benzidine hydrochloride. When such slides are gassed with water vapor, the slide is seen to be covered with small, uniformly dispersed crystals of the sulfates. Crystals formed from samples taken simultaneously on the two types of slides are alike under ordinary illumination, but under polarized light only the benzidine sulfate crystals are visible. A film of glycerol on a microslide containing benzidine hydrochloride in solution reacts very rapidly with air-borne sulfates. Gassing with water vapor aids the formation of crystals, which are free to move. At the same time there is a tendency for larger crystals to form (Figure 4).

Figure 7. Reaction of air-borne zinc oxide with acetyl acetone

Pure sulfates, including such substances as gypsum, anhydrous calcium sulfate, sodium sulfate, ammonium sulfate, and liquid sulfuric acid, all produced benzidine sulfate crystals fairly readily. Microgram amounts of nonsulfate materials such as commonly occurring oxides, carbonates, and chlorides do not produce these crystals.

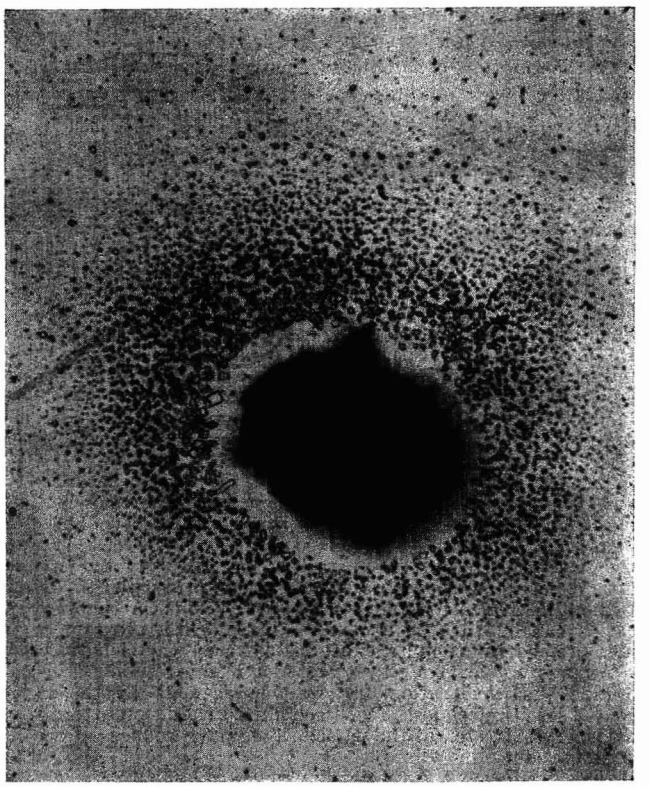


Figure 8. Reaction of alumina particle with acetylacetone

Organic Material. Samples of air-borne dust (50 to 100 mg.) obtained from high-volume samplers were placed on microslides on a platinum heater and exposed to heat in an atmosphere of nitrogen. With a fixed temperature setting the chamber was swept with nitrogen for 30 minutes, and the organic material evolved was caught on silica gel in absorbers downstream. The adsorbent is changed, the temperature setting is raised, and the procedure is repeated with fresh silica gel. In this way a certain amount of separation of the organic constituents is achieved. The various fractions on silica gel are eluted with appropriate solvents such as iso-octane or cyclohexane and the ultraviolet transmittance of the solution is measured. After the ultraviolet spectral transmittance curves have been prepared, the samples are subjected to investigation in the infrared wave lengths. Identification of the substances producing these curves is proceeding.

Metallic Oxides and Metals. Included in this category for the sake of simplicity are lead, zinc, cadmium, antimony, copper, manganese, beryllium, and uranium. These substances under the microscope behave as amorphous particles. Successful gaseous reactions produce crystalline substances. Gassing with hydrochloric acid produces droplets of the chlorides, which are hygroscopic except for lead and cadmium chlorides. The reaction between cadmium oxide and hydrochloric acid is rapid and distinctive (Figure 5). It is evident that the crystals in this case were formed by the evaporation of a droplet.

After hydrochloric treatment the metal chlorides can be exposed to hot acetylacetone vapor. The reaction of calcium

chloride with acetylacetone has been mentioned above. Similarly, cupric oxide, cadmium oxide, manganese dioxide, and zinc oxide react rapidly with acetylacetone after prior treatment with hydrochloric acid vapor and produced well-formed crystals. Zinc oxide and metallic zinc will react with acetylacetone without the need for preliminary acid treatment (Figure 6). The crystals shown in Figure 6 were formed from powdered zinc oxide reacting with acetylacetone. In 30 minutes or less, no zinc oxide is left unconverted. Figure 7 shows the same crystals formed from zinc present in an air-borne dust sample.

As the resistance of alumina to chemical attack is well known, it was interesting to note the reaction of acetylacetone vapor with alumina particles (Figure 8).

DISCUSSION

If allowances are made for certain limitations, the gaseous reactions described can be of help in identifying air pollutants. As a guide to the investigator, the maximum amount of information about the sample should be obtained before investigation. From a consideration of the sampling location, the probable composition of the air sample may be predicted. This should influence the method of sampling and the gassing treatment used. In such investigations, much time can be spent without result unless at the outset it is decided the investigation of the sample will be along restricted lines. We do not look for organic material in dust from a hard rock gold mine; on the other hand, air samples from many industrial cities are largely organic and should be treated accordingly.

Because organic substances are not too reactive chemically, and show no structure under the microscope, air samples containing much organic material are best taken with high-volume samplers on glass fiber filters. Such glass filters can be heated before use to ensure freedom from organic material, and the sample, after collection, can be exposed to heat and acid vapor treatment without interference from the sampling medium. Actual identification can be done by a combination of chromatography and measurements in the ultraviolet and infrared wave lengths.

As it is not always easy to restrict reaction products to the original particle location, improvement is made by the use of coated sensitized slides. Sensitized films, in addition to localizing halos, crystals, etc., at the particle site, may produce colored reaction products in favorable cases.

Identification by crystal form alone is difficult, as crystal habit may vary with crystallizing conditions. It is desirable, therefore, to develop crystal reactions producing reproducible and characteristic crystal forms, without the need for too precise control of conditions. The cadmium chloride crystals formed from cadmium oxide are an example of a suitable reaction, easily and consistently produced, and characteristic in form.

To be of practical use, a reaction must be reasonably rapid, unless it possesses some other advantage such as specificity. The cadmium chloride reaction meets this requirement as well.

Identification based on tests carried out on the effluent gas stream may be more sensitive than visual indications given under the microscope, or may be the only indication of a substance sought. This is condition 3, illustrated by experience with arsenic.

It is not necessarily desirable that these gas phase reactions take place only at the particle site. The huge size of some of the benzidine sulfate crystals formed may be due to the diffusion of sulfite ions to a central nucleus. Where magnification by halo formation or by diffusion to a common center occurs, submicroscopic particles may be made visible.

ACKNOWLEDGMENT

The help of T. F. Doherty, who prepared the drawings, George Ensell, who made the glassware, and Morris Katz and Ellis Kerr, who made arrangements for the photographic reproduction, is hereby acknowledged.

LITERATURE CITED

(1) Cadle, R. D., ANAL. CHEM., 23, 196 (1951).
(2) Cadle, R. D., Rubin, S., Glassbrook, C. I., and Magill, P. L., *Arch. Ind. Hyg. Occupational Med.*, 2, 698–715 (1950).
(3) Foster, W. D., and Schrenk, H. H., U. S. Dept. Interior, Bur. Mines, Rept. Invest. 3368 (1938).
(4) Gitzen, W. H., ANAL. CHEM., 20, 265 (1948).
(5) Lennox, D. H., and Leroux, J., *Arch. Ind. Hyg. Occupational Med.*, 8, 359–70 (1953).
(6) Leroux, J., "Contribution à l'Étude des Poussières Atmosphériques par Diffraction des Rayons-X et Microscopie Électronique," thesis, Faculté des Sciences, Université de Paris, June 1954.
(7) Shore, V. C., and Katz, M., Defense Research Chemical Laboratories Report 154, 15th Annual Meeting, American Industrial Hygiene Association, Chicago, 1954.
(8) Strebinger, R., and Orth, E., *Monatsh.*, 80, 254–9 (1949).
(9) West, P. W., and Granatelli, L., ANAL. CHEM., 24, 870 (1952).
(10) Williams, C. R., *J. Ind. Hyg. Toxicol.*, 19, 44–52 (1937).

RECEIVED for review September 3, 1954. Accepted March 15, 1955.

Instrumental Measurements of Visibility in Air Pollution Studies

GEORGE D. CLAYTON[1] and PAUL M. GIEVER[2]

U. S. Public Health Service, Detroit, Mich.

An instrument which measures transmittance (the Transmissometer) was found to be a useful tool, in air pollution studies, for measuring visibility in an urban atmosphere. It was relatively free of operating difficulties, required little attention, and was sufficiently sensitive to measure instantaneous variations in visibility. In a study of the relationship among data obtained with the instrument, the soiling power of the atmosphere, and the mass weight of air-borne particulates, it was found that there was no correlation among these three different methods of measuring aerosols. Visibility measurement therefore cannot be used as an index of the soiling power of the atmosphere or the mass weight of the air-borne particulates. Rain removed from the atmosphere some aerosols which cause discoloration, while snow had no such effect.

THE adverse effects of aerosols on visibility have been recorded since the industrial age. As early as the thirteenth century, objections were raised over the burning of coal and its effect on visibility in London. During the known air pollution disasters such as Meuse Valley, Donora, and London, the reductions in visibility were reported to be the greatest during the periods when most of the deaths were occurring. In Los Angeles there is reported a definite relationship between smog and visibility—e.g., the greater the smog and eye irritation, the greater is the reduction in visibility.

Reduced visibility plays an important role in other incidents of a tragic nature, such as aviation, automotive, and vessel traffic accidents.

In addition to the safety and health problems, poor visibility

[1] Present address, consulting engineer, 14125 Prevost St., Detroit 27, Mich.
[2] Present address, Detroit Edison Co., Detroit, Mich.

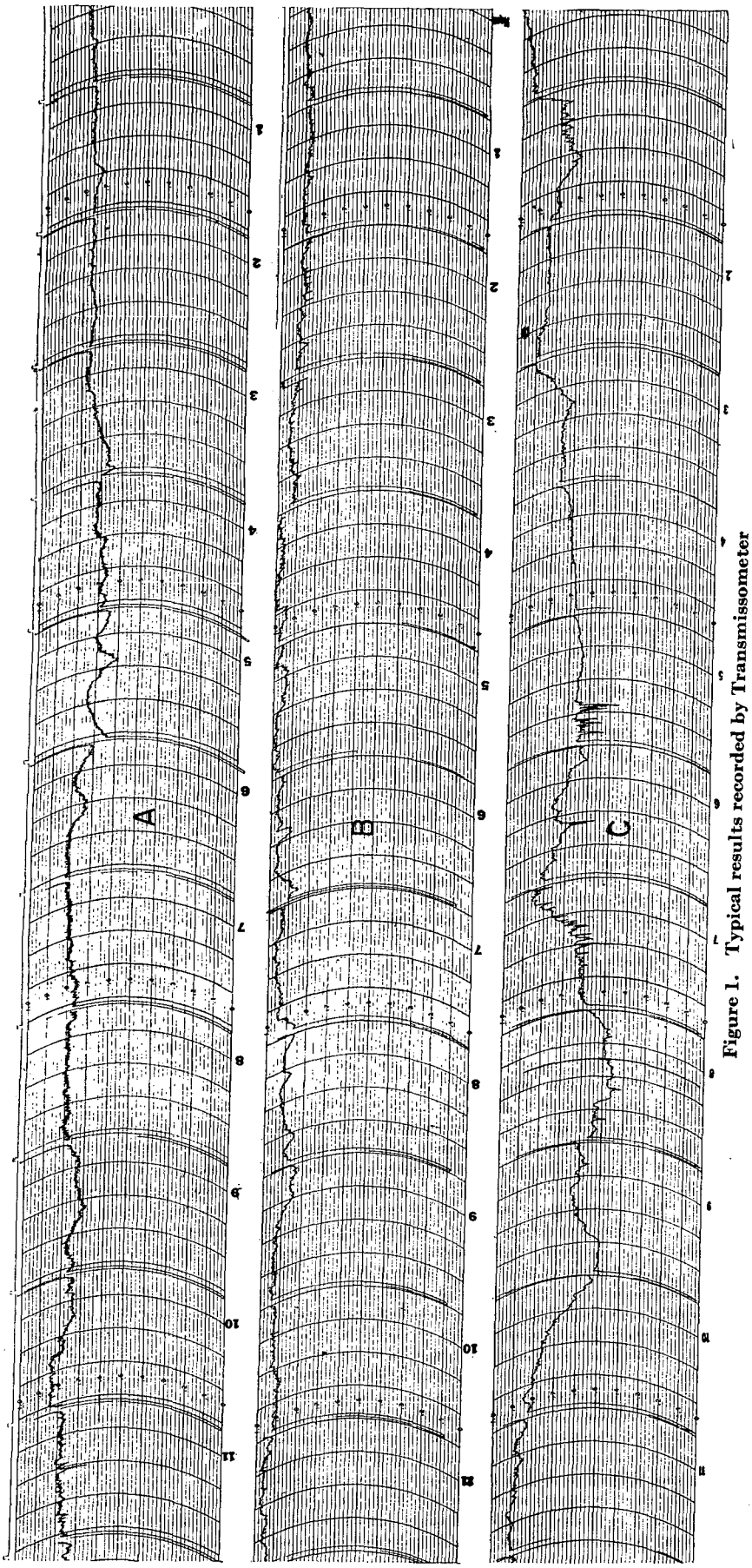


Figure 1. Typical results recorded by Transmissometer
A. Typical day
B. Exceptionally clear day
C. Day showing heavy pollution from 7 A.M. to 9:30 P.M.

has a decided economic effect on an urban area. The cost to the aviation industry alone because of "closed" airports is considerable.

The majority of air pollution ordinances in the various communities of the United States have been inaugurated as a result of the observed reduced visibility created by air pollution. From these few observations, it may be seen that reduced visibility has been associated with health, safety, economy, and the general esthetics of a community. It therefore becomes an important facet in any comprehensive study of air pollution.

It is recognized by scientists and the public alike that weather conditions that result in fog, snow, and rain also have an important effect on visibility, and it is known that condensation nuclei which affect visibility are formed by nature as well as community activities.

This presentation discusses the effect of meteorological phenomena and atmospheric particulates upon visibility.

In the measurement of visibility it was first necessary to find an instrument that would accurately record atmospheric transmittance during an entire 24-hour period. The Transmissometer. developed by C. A. Douglas and associates of the National Bureau of Standards, U. S. Department of Commerce, Washington, D. C., met requirements and was selected as meriting experimentation in atmospheric pollution studies. Douglas cooperated completely in the endeavor, by supplying an instrument, inspecting and giving final approval of the location of the installation, and visiting Detroit during the several months that the instrument was being operated.

DESCRIPTION OF INSTRUMENTS

The Transmissometer was designed to measure visibility by light transmittance. It consists of a 350,000-candle power light source, a phototube receiver, an amplifier, and an indicator. The distance between the points of transmittance and reception may be up to 1.5 km. The output of the receiver is transmitted to the indicator and the recorder, which may be located several miles from the actual site of the testing.

The intensity of the light is controlled by a voltage-regulating transformer and rheostats. A zero check is made each hour by an automatic cutoff and checks may be made at the recorder as indicated or desired. The expected service of a lamp is from 3 to 6 months.

The receiver unit consists of a lens, a diaphragm, a photopulse unit, and an amplifier. The light, focused by

means of the lens on a pinhole in the diaphragm, strikes the phototube receiver and there generates pulses the frequency of which is directly proportional to the intensity of the light on the phototube. These pulses are amplified and transmitted to the indicator.

In the pulse generator unit, current charges a capacitor with sufficient voltage to cause a discharge through a neon lamp. The capacitor rapidly discharges through the neon lamp and a resistor until the voltage is no longer sufficient to maintain a current through the lamp. The voltage drop across the resistor supplies a voltage pulse to the grid of a 6J5 tube, causing a momentary change in the plate current of the tube. The resulting momentary change in voltage drop across a transformer, when amplified, supplies the pulse signal which is received at the indicator.

The indicator consists of a frequency measuring unit, a two-stage amplifier, and a calibrator. The frequency meter levels and averages the pulses which are received through the amplifier and produces a meter reading which is directly proportional to the pulse frequency and, therefore, to the transmittance of the atmosphere between the receiver and the light source.

A recorder connected to the indicator gives a continuous record of the indicator meter reading on chart paper marked to show percentage of light transmittance by time of day.

The installation in Detroit was approximately 125 feet above ground level with approximately 800 feet between the light source and the receiver. The light source was mounted on the roof of the 10-story Federal Building and the receiver was located on the tenth floor of the Majestic Building, both in the center of the business district of Detroit.

A high-volume air sampler was used to collect particulate matter. The essential parts of the sampler consist of a vacuum cleaner–type blower mounted in an aluminum housing, a filter paper retaining ring located on the intake side of the blower, and a U-tube manometer located on the exhaust side. The manometer was used for determining pressure differential between the inside of the housing and the atmosphere. The manometer was calibrated with a dry gas meter to obtain the air flow in cubic feet per minute.

An accordion-pleated filter paper, presenting a large surface, was used for collecting air-borne particulate matter. The rate of flow through the average clean filter was approximately 65 cubic feet per minute. As the sample was being collected, the rate of air flow decreased as a result of resistance caused by particulates on the filter. In areas of heavy pollution, at the end of a 24-hour sampling period, the flow rate often was reduced to as low as 25 cubic feet of air per minute.

In operation the filter was replaced every 24 hours. Manometer readings were taken before the sampler was stopped for removal of the filter, and immediately after a clean filter was inserted. To increase accuracy in the manometer reading, instructions were given to stop and start the instrument three times (allowing a minimum of 1 minute of operation to ensure that the liquid in the manometer reached equilibrium) and a reading was taken in each instance. The average of the three readings was then recorded.

The filter was weighed in a manner developed by Byers and Keenan (*1*).

The Wilson automatic filter paper sampler utilizes a clock mechanism which operates an "on and off" switch. Air is drawn through a circle of filter paper, 0.5 inch in diameter, held by an automatic clamp. The sampling rate was 10 liters of air per minute in 25 minutes of each hour. In operation, one 25-minute sample is obtained during a 60-minute cycle, at which time the paper strip is automatically moved to a new position for the next sample.

The stains obtained with this instrument were evaluated with a reflectometer using a blue light filter with a dominant length of 400 mμ. The instrument was set at 100% reflectance for the paper being used and a check of that setting was made after each sixth reading, in order to minimize inaccuracies due to the varying reflecting quality of the paper. The stain was placed over the detector element and percentage of reflectance read on

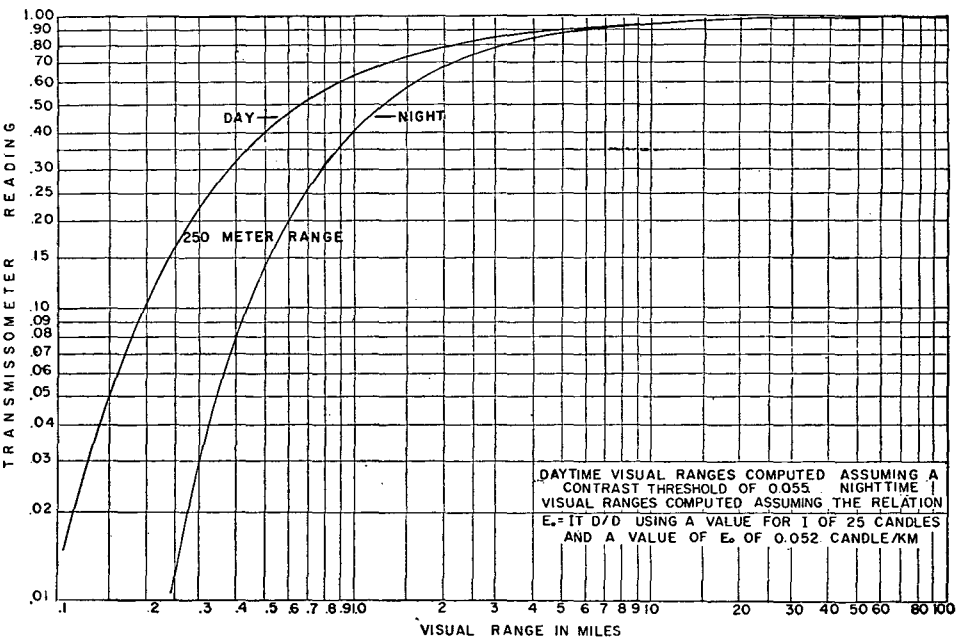


Figure 2. Visual range chart

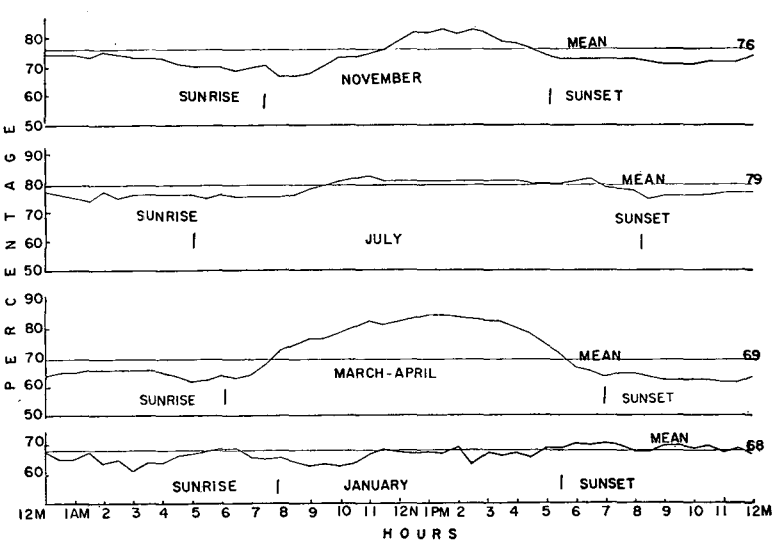


Figure 3. Mean half-hour light transmittance for selected months, representing four seasons

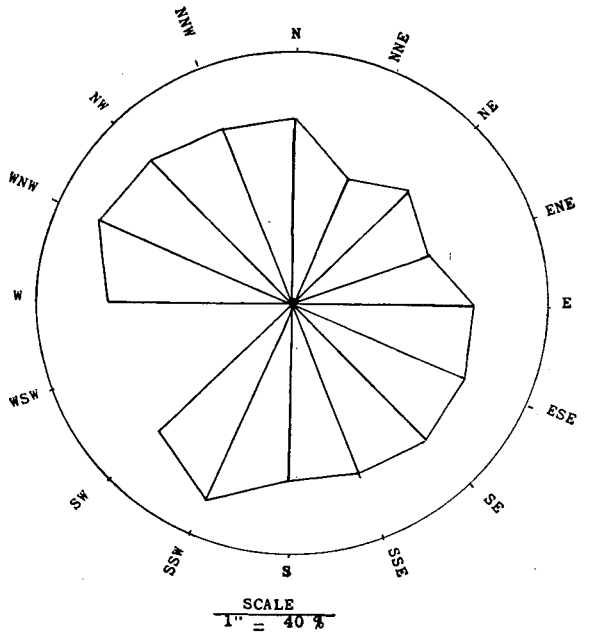


Figure 4. Influence of wind direction on light transmittance

the indicator. The instrument had very little lag and readings were reproducible to within 0.2%.

RESULTS OF EXPERIMENT

Figure 1 shows typical records obtained with the Transmissometer on days of varying intensity of light transmittance. On a very clear day the transmittance record is relatively constant, while on days of lower light transmittance values, greater variations and more oscillations occurred. This figure further shows the clarity of record and sensitivity of the instrument to variation in transmitted light.

Figure 2 is the visual range chart prepared by Douglas for converting the Transmissometer readings into visibility. There are two curves: one for daytime readings and one for nighttime readings. This was necessary because of the day and night definitions of visibility (*4*).

The instrument is most sensitive in the 0.1- to 1.0-mile range, with good sensitivity up to 4 miles.

In Figure 3 is shown the average light transmittance by the half hour for four seasonal months. The mean value given for each of the seasons was obtained by averaging the daily results for the period of observation. The monthly mean values were lower in the winter and spring than during the summer and fall. This is especially interesting, because the high-volume air-sampling studies, which give the total weight of air-borne particulates, show higher mass weights in the summer and fall than during the winter and spring.

To determine if the resulting lower transmittance data were due to particulates or snow and rain, the amounts of precipitation were studied. There was somewhat more snow in January and March–April than during November, and, of course, no snow in July. The total precipitation for the four seasons was: January, 1.9 inches; March–April, 5.7 inches; July, 3.1 inches; and November, 0.8 inch. After due consideration of these factors it is believed that the lower transmittance values obtained in January and March–April were due to aerosols rather than snow and rain.

A further study of Figure 3 shows the diurnal and annual variations of light transmittance. In January there was very little variation in the mean light transmittance in 24 hours. In March–April, light transmittance increased considerably from 8:00 A.M. to 5:00 P.M. In July the mean light transmittance was slightly higher than for the other months plotted and the values obtained for the 24 hours were rather constant. In November, the transmittance increased for a short time between noon and 4:00 P.M.

Sutton (*3*) mentions other climatic factors which influence the diurnal variation of lapse rates, and in the case of Detroit, tend further to differentiate between summer and winter conditions. Sutton points out that under overcast and windy conditions the diurnal variation of the lapse rate almost completely disappears. During December and January in Detroit, mean cloud cover is 75%, whereas in June through September it is only 50%. Mean surface wind speed in December and January is 11.8 miles per hour, whereas in June through September it is only 9.2 miles per hour. The greater frequency of cloudy, windy weather in winter will tend further to diminish the diurnal variations of lapse rate and hence of particulate pollution.

In Figure 4 is shown the influence of wind direction on light transmittance. Light transmittance values on days of more than 0.15 inch of rain or other obvious meteorological conditions which caused reduction in visibility were not used in compiling the data for Figure 4. The lowest light transmittance was obtained with winds from the north northeast through east northeast. In these sectors are a large number of small and large industries extending for several miles, as well as a large number of residents. Unfortunately, north northeast and east northeast values represent only 1 day, and only 3 days are represented by the northeast direction. Therefore, less significance should be attributed to these values than to the subsequent ones which represent the average of a larger number of readings. When the wind was from the east the visibility improved. In this direction lay the Detroit River and Lake St. Clair.

Visibility improved considerably when the wind was from the south southwest. In this direction lay the Detroit River, the

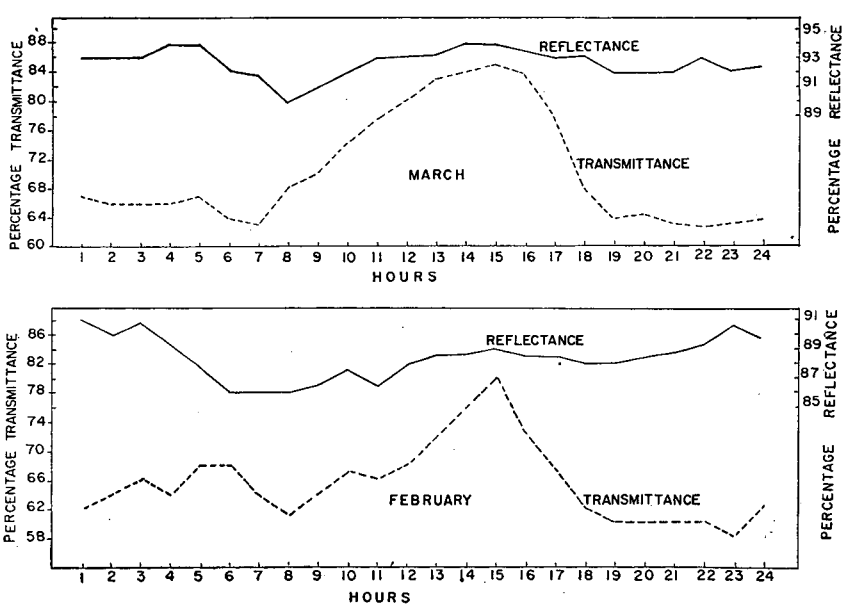


Figure 5. Relation of hourly median reflectance of filter paper samples to hourly median transmittance readings

residential section of Windsor, and, farther away, a truck farming area. As the wind moved in the southwest direction, visibility decreased, which may be accounted for by the large number of industries in that area.

The greatest visibility occurred when the wind was from the west through north northwest. In this direction lay mostly homes and small business establishments. The primary reason for the greater visibility from this direction, however, is that these winds are usually associated with outbreaks of fresh, clear polar air.

Figure 5 shows the relationship of reflectance values to transmittance values. These data were obtained by finding the median value by the hour for the months of February and March. Tabulation of a large number of figures for each hour of the month aided in removing any bias, thus permitting a more accurate appraisal of the relationship between the two sets of data. In addition to method of presentation in Figure 5, the data were also treated statistically and found to have a correlation coefficient of 0.365.

It is apparent from Figure 5 and the correlation coefficient that there is little correlation between the two sets of data. This is expected, as the reflectance factor is primarily a measure of tars and carbon, as well as other colored material in the atmosphere, and the light transmittance is affected by all aerosols, their numbers, size, and shape, as well as the aerosol's refractive and absorption indices.

The Transmissometer showed greater fluctuation than did the filter paper sampler, thus indicating its greater sensitivity.

In some preliminary work, Katz and others (*2*) reported that temperature anomaly had an effect on air pollution in the Detroit area. As the temperature anomaly increased, the mass weight of the aerosols increased; conversely, as the temperature anomaly decreased, the mass weight of the aerosols decreased. To determine the influence of temperature anomaly on the Transmissometer and the tape recorder, data representing both minimum and maximum temperature anomalies were selected and are presented in Figure 6. March 12 and 13 were selected as two days similar in respect to wind direction, wind speed, and rain. The only difference was the temperature anomaly. March 10 and 30 were similarly selected. In the following table are presented the median values for the days under study.

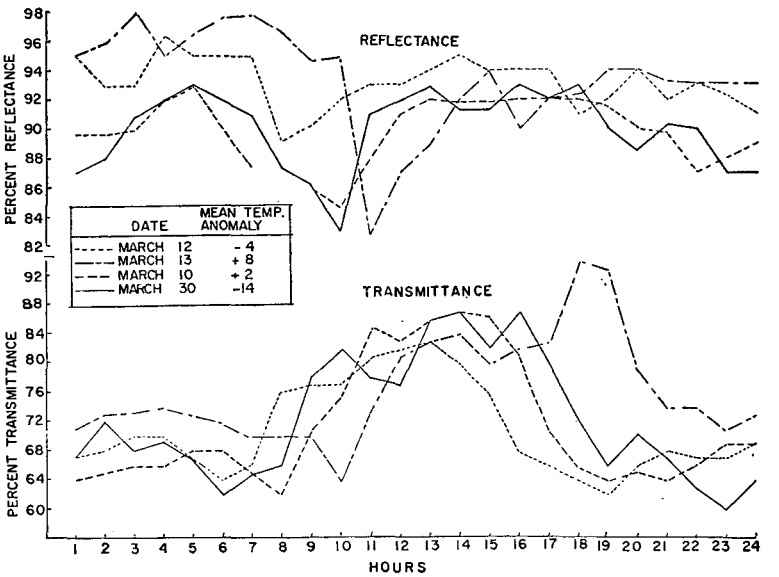


Figure 6. Influence of temperature on light transmittance and reflectance from filter paper samples by time of day

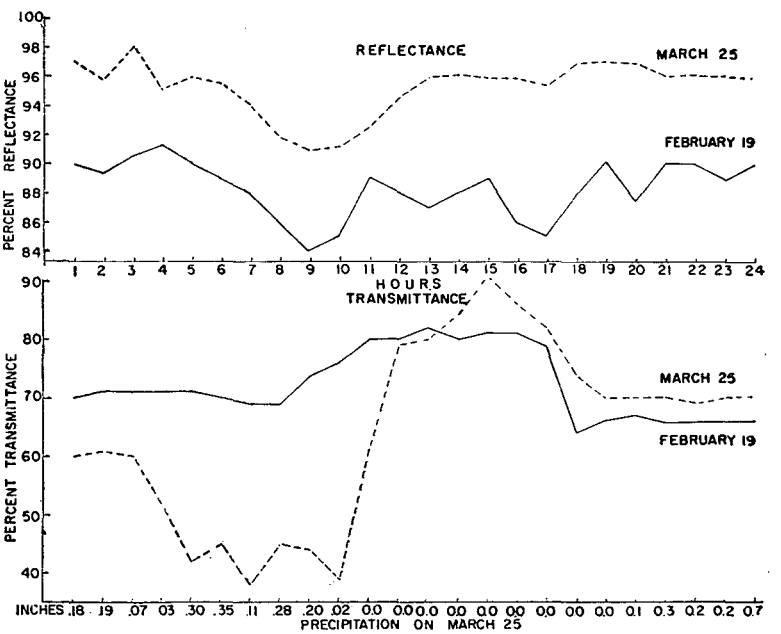


Figure 7. Light transmittance of filter paper samples for rainy day (March 25) and day of no rain (February 19)

Median Transmittance and Reflectance Values for Selected Days

Day	Mean Temp. Anomaly, °	% Median Transmittance	% Median Reflectance
March 10	+ 2	70	90.2
March 30	−14	70	91.0
March 12	− 4	70	93.3
March 13	+ 8	74	93.8

A study of Figure 6 and the table reveals that the mean temperature anomaly has little or no effect on the percentage of transmittance and reflectance. However, the data do indicate that on individual days there may be a fair correlation between transmittance and reflectance. At least the morning troughs of reflectance seem to follow those of transmittance with a lag of about 2 hours.

There has been much discussion regarding the effect of rain on cleansing the atmosphere. Figure 7 presents the transmittance and reflectance data collected on a day of rain and a day having no rain. These days were selected after due consideration of wind direction, speed, and other meteorological factors. As would be expected, there was a decrease in light transmittance during periods of very heavy rain, as noted between the hours from 5:00 to 10:00 A.M. on March 25. As the rain ceased, the visibility improved rapidly, reaching a maximum greater than that for the day of no rain. The reflectance follows a similar pattern for that day; during the heaviest rain, the reflectance was less, indicating the atmosphere was dirtier. This behavior would suggest that

the rain precipitated the particles, and if the rain persisted for a sufficient length of time, there was cleansing of the atmosphere. As the reflectance factors for March 25 were constantly greater than on February 19, rain does reduce the soiling power of the aerosols.

The cleansing effect of snow upon the atmosphere has also been a matter of much conjecture. In Figure 8 are shown the transmittance and reflectance data collected on a day of snow, March 29, and a day of no snow, March 31. All other meteorological factors on these two days were similar. As would be expected, snow considerably decreased visibility. However, it had little or no effect upon those aerosols which cause discoloration of the filter paper. Even during the periods of heavy snow and low Transmissometer readings, the reflectance factors remain essentially the same.

Figure 9 shows the relationship of light transmittance, total weight of air-borne particulates, and light reflection from filter paper samples. For this test, a Wilson tape recorder and a high-volume air sampler were located next to the receiver of the Transmissometer.

The filter of the high-volume air sampler was changed every 24 hours, and the weight of particulate matter was determined for the period. Data from the tape recorder and Transmissometer were tabulated and a median figure was obtained to compare with the total weight data.

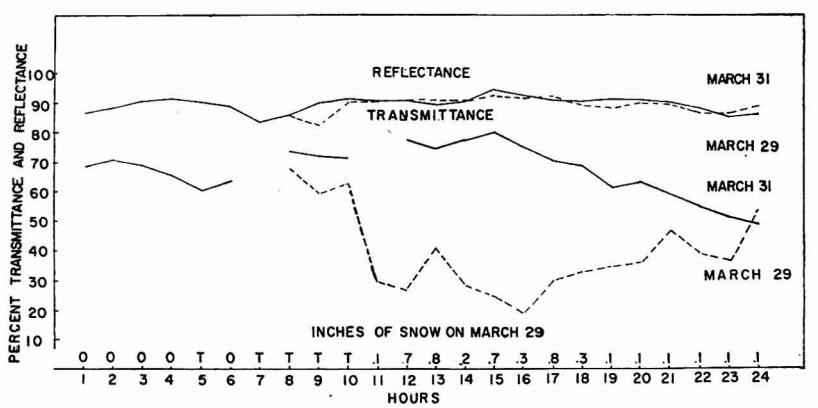


Figure 8. Comparison of light transmittance and filter paper sample reflectance factors on day of snow (March 29) to day of no snow (March 31)

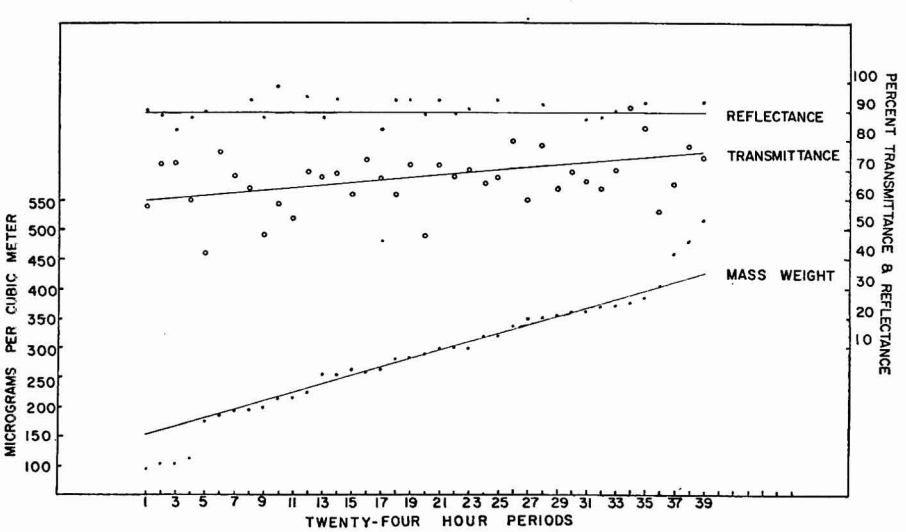


Figure 9. Relation among weight of air-borne particulates, transmissivity, and reflectance from filter paper samples

There is little or no agreement between the data obtained by these three instruments. A Transmissometer measurement which records visibility cannot be used to determine either the weight of suspended aerosols or the color of the aerosols. Neither can the reflectance of a filter tape give a total weight of the aerosols nor a value for visibility. This indicates that, for a comprehensive study, all three instruments must be used.

These findings may be explained as follows: The high-volume air sampler collects on a filtering pad the mass of air-borne particulates, and it is so reported. From the tape recorder are obtained the reflectance data which record the discoloration of aerosols and are greatly influenced by the tars and carbon particles therein. The Transmissometer measures the transmissivity of the air. It is known that the reason for the reduction in visibility is not formation of a barrier by the particles, but rather the phenomenon of light scattering. The number of particles per unit volume in an aerosol is very great, although the mass concentration in comparison with gas contaminants may be very small. An urban atmosphere is composed of particulates ranging in size from near the lower range of the wave length of visible light (blue haze noted in many cities) to over 40 microns. This may be the reason for the poor correlation between light transmittance values and total weight.

CONCLUSIONS

Sensitivity of the Transmissometer is adequate for measuring small variation in light transmittance.

Visibility was greater in Detroit in the summer and fall than in the spring and winter. Light rain did not appreciably affect light transmittance, while heavy snows and rain caused a marked reduction. No relationship was found to exist between reflectance values of filter paper spot tests and visibility.

Temperature anomalies had little or no effect on visibility or the aerosols that cause discoloration.

Heavy persistent rain reduces the aerosols that cause discoloration. Snow does not reduce the aerosols that cause discoloration. No relationship was found to exist among visibility, mass weight, and soiling power of aerosols.

ACKNOWLEDGMENT

The authors wish to acknowledge the cooperation of G. A. Cave, Officer in Charge, Windsor Laboratories, International Joint Commission, Windsor, Ontario, Canada, who performed the necessary laboratory work, and Howard Baynton, meteorologist, Meteorology Service of Canada, Windsor Laboratories, International Joint Commission, Windsor, Ontario, Canada.

LITERATURE CITED

(1) Byers, D. H., and Keenan, R. G., *Arch. Ind. Hyg. Occupational Med.*, 6, 226–30 (1952).
(2) Katz, M., Cave, G. A., and Baynton, H. W., International Joint Commission, Ottawa, Ontario, Canada, Progress Rept. 7 (1953).
(3) Sutton, O. G., "Micrometeorology," p. 190, McGraw-Hill, New York, 1953.
(4) Theissen, A. H., "Weather Glossary," U. S. Weather Bureau, Washington 25, D. C., 1942.

RECEIVED for review November 1, 1954. Accepted March 16, 1955.

Water-Containing Jars and Greased Plates for Dustfall Measurements

RICHARD L. POND[1] and R. R. PAXTON

Department of Chemistry and Chemical Engineering, Stanford University, Stanford, Calif.

In a comparison of two methods for measuring fall out of dust from the atmosphere, cylindrical glass jars partially filled with distilled water were exposed beside 5 × 10 inch glass plates coated with Vaseline, on small platforms 18 feet above an open field. After exposure, the water from the jars was analyzed for total solids, both soluble and insoluble. The grease and dust were removed from the plates, using a petroleum ether-benzene solution, the resultant slurry was filtered through a sintered crucible, and hydrocarbon-insoluble dust was weighed in both an oven-dry and an ignited state. The standard deviation between concurrently exposed water jars was 12.6%. The comparable value between greased plates was 6.8%. Thus the greased plate procedure gave more reproducible results and, with but one exception, slightly higher dustfall rates.

THE purpose of this investigation was to ascertain the reproducibility of dustfall measurements made by water-containing jars and by greased plates, and to compare the two methods.

METHODS

Water-Containing Jar Method (*1*). Glass battery jars $6^{1}/_{16}$ inches in inside diameter and 8 inches deep were used. Each jar was filled to a depth of about 2.25 inches with 1 liter of distilled water, and more water was added as needed to prevent the jar from becoming dry during the exposure period. A blank was prepared by adding water to a similar but tightly covered jar.

After exposure, gross contaminants such as insects, leaves, or twigs were removed from the jar, and the water was filtered through a sintered crucible (Selas No. 4001). The insoluble material retained in the filter crucible was dried at 105° C., weighed, ignited at 950° C., and weighed again. The filtrate was evaporated to dryness and the residue was weighed, ignited at 950° C., and weighed again.

The total solids value used to compute the dustfall rate was the sum of the insoluble and soluble residues. This sum, typically 10 to 20 mg. for a week's exposure, was first corrected for the blank, which generally varied from 2 to 8 mg.

Greased Plate Method (*2, 3*). Rectangles of window glass (5 by 10 inches) were coated by painting them with a solution (6 grams per 100 ml.) of Vaseline in petroleum ether (boiling point 55° to 85° C.). The solvent evaporated in several seconds and left a coating about 4 microns thick.

After exposure, any portions of the greased surface marred in handling or by trapped insects were cleared with a razor blade. The relatively small area thus cleared was subtracted from the gross area covered with grease and dust.

The collected dust was separated from the Vaseline by dissolving the coating from the plates in a solvent containing 75% petroleum ether (77° to 110° C.), 20% benzene, and 5% Cellosolve, and filtering the mixture through a sintered crucible (Selas 2001). (Less than 2% of dust was soluble in this solvent.) The dust was weighed in the Selas crucible in both an oven-dry and an ignited condition.

A sample consisted of the dust from four plates having a nominal exposed surface of 200 square inches. The blank was the dust from four coated plates stored in the sealed carrying case during the exposure period. A typical value of the blank was 0.9 mg., whereas the gross sample weighed about 25 mg. (1-week exposure, oven-dry basis).

Obtaining Dust Samples. Several successive groups of dustfall measurements were made, all during mild rain-free weather. Coated plates were transported to the exposure site in closed plywood carrying cases which supported the plates by their edges (Figure 1). The plates were set out in rectangular platforms just large enough to hold eight plates. All platforms were equipped with brass wires mounted around their periphery to protect the plates and jars from birds (Figure 2). Exposure platforms were mounted at the top of an 18-foot tower in an open field (Figure 3). Some plates and jars were exposed on a platform 5 feet high.

All reported dustfall rates were corrected for the blank and represent net values. The calculated net values are based on the weight of dust collected, length of exposure period, and area of collecting surface (*4*).

Table I. Reproducibility of Water Jar Method

(Ignited dustfall rates)

Exposure		Dustfall, Lb./Acre-Day			
Date	Duration, days	Individual	Av.	Dev.	% Dev.
Aug. 6 to 11	4.86	0.230 0.220	0.225	0.005	2.2
Aug. 11 to 17	5.72	0.196 0.171	0.183	0.013	7.1
Jan. 30 to Feb. 4	5.04	0.346 0.328	0.337	0.009	2.7
Feb. 22 to 24	2.18	0.171 0.121	0.146	0.025	17.1
Apr. 10 to 13	3.06	0.366 0.320	0.343	0.023	6.7

Standard deviation, 12.6%

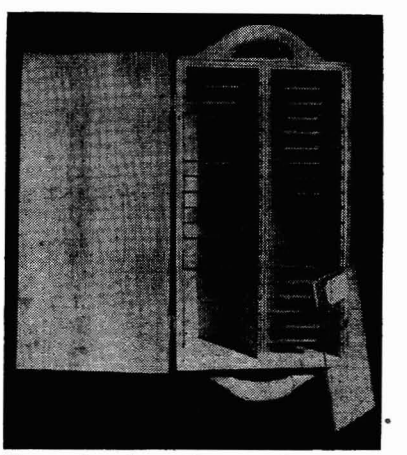


Figure 1. Carrying case for plates

REPRODUCIBILITY

Water Jar Method. Table I shows five pairs of dustfall rates obtained using water-containing jars, which were exposed concurrently at the same level and within 4 feet of each other. The per cent deviation for each pair is based on the deviation of each jar from the average of the two dustfall rates.

Greased Plate Method. Table II shows seven groups of dustfall rates obtained concurrently by the greased plate method. As in Table I, the per cent deviations are based on the average dustfall rate for the group. Deviations ranged from 0.9 to 9.7%,

[1] Present address, California Research Corp., Richmond, Calif.

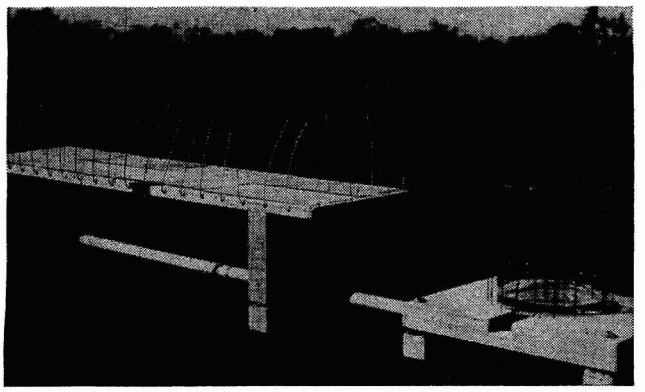


Figure 2. Plate and jar platforms

Wires guard against bird damage

the standard deviation being 6.8%. These deviations were compared to those reported in Table I using the statistical F test (5). It was found that the greased plate method has a better precision than the water jar method at the 95% confidence level.

Table III reports data for the same tests as Table II, but on an oven-dry basis. When oven-dry rather than ignited weights are used, the deviations range from 0.2 to 10.8%, the standard deviation being 7.0%. Simple drying of the residues instead of igniting them resulted in no significant loss of precision.

Table II. Reproducibility of Greased Plate Method

(Ignited dustfall rates)

Exposure		Dustfall, Lb./Acre-Day			
Date	Duration, days	Individual	Av.	Dev.	% Dev.
March 26 to Apr. 9	14.00	0.320 0.314	0.317	0.003	0.9
May 4–6 8–19	12.94	0.248 0.205	0.227	0.022	9.7
July 28 to Aug. 3	6.15	0.217 0.225	0.221	0.004	1.8
Aug. 6 to 11	4.86	0.270 0.291	0.280	0.011	3.9
Aug. 11 to 17	5.72	0.336 0.324	0.330	0.006	1.8
Feb. 22 to 24	2.18	0.197 0.190 0.216 0.228	0.208	0.011 0.018 0.008 0.020	5.3 8.7 3.8 9.6
Apr. 10 to 13	3.06	0.386 0.450 0.439	0.425	0.039 0.025 0.014	9.2 5.9 3.3

Standard deviation, 6.8%

For all three sets of data, the per cent deviation tends to be smaller for longer exposures. The one real exception to this trend is the 12.94-day exposure with greased plates—the only one that was interrupted by a few days of rain.

COMPARISON OF METHODS

In six measurements of the dustfall rate greased plates were exposed concurrently and beside water jars. In most instances the test array included at least two sets of plates and two water jars. Table IV shows the averaged dustfall rate found by each of these two methods. In five out of six cases the average rate found by the greased plate method was higher than that found using water jars. The weather during the test period of this sixth sample was unusual, in that it included several damp, foggy days. When these plates were demounted from the test platform there was water beneath them, and a brown stain was left on the platform marking the edges of the plates. This did not occur in any of the other five tests.

A statistical examination of these five sets of data showed that at the 99.9+% confidence level, the greased plate method gives higher dustfall rates.

DISCUSSION

Greased plates have been widely used to measure dustfall rates, particularly in the western United States, where the long dry summers favor their use. Practical experience has shown that the greased plate method has the following advantages: relatively large samples are obtained, handling in the field is simplified (no liquids to spill), individual dust particles can be examined microscopically, there is no evaporation, freezing, or mold growth in the solvent, and contamination of the sample (by bird droppings, etc.) is readily detected.

This investigation has confirmed these advantages, and has shown that under the conditions of the test, the greased plate method gives more reproducible dustfall measurements than the

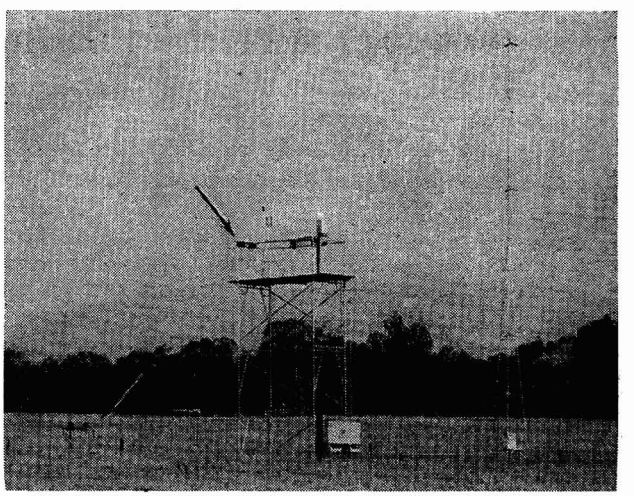


Figure 3. Test site

Plates and jars exposed on upper and lower platforms

Table III. Reproducibility of Greased Plate Method

(Oven-dry basis)

Exposure		Dustfall, Lb./Acre-Day			
Date	Duration, days	Individual	Av.	Dev.	% Dev.
Mar. 26 to Apr. 9	14.0	0.451 0.453	0.452	0.001	0.2
May 4–6 8–19	12.94	0.371 0.305	0.338	0.033	9.8
July 28 to Aug. 3	6.15	0.293 0.276	0.284	0.0085	3.0
Aug. 6 to 11	4.86	0.390 0.397	0.393	0.0035	0.9
Aug. 11 to 17	5.72	0.410 0.395	0.402	0.0075	1.9
Jan. 30 to Feb. 4	5.04	0.250 0.297 0.276 0.295	0.279	0.030 0.020 0.004 0.016	10.8 7.2 1.5 5.7
Feb. 22 to 24	2.18	0.294 0.289 0.289 0.333	0.301	0.007 0.012 0.012 0.032	2.3 4.0 4.0 10.6
Apr. 10 to 13	3.06	0.641 0.761 0.752	0.718	0.077 0.043 0.034	10.7 6.0 4.7

Standard deviation, 7.0%

Table IV. Comparison of Two Methods
(Ignited weights)

Exposure		Av. Dustfall, Lb./Acre-Day	
Date	Duration, days	Water jar	Greased plate
July 28 to Aug. 3	6.15	0.217	0.221
Aug. 6 to 11	4.86	0.225	0.280
Aug. 11 to 17	5.72	0.183	0.330
Jan. 30 to Feb. 4	5.04	0.337	(0.181)
Feb. 22 to 24	2.18	0.146	0.208
Apr. 10 to 13	3.06	0.343	0.425

water-jar method. Finally, in the absence of rain or damp fog, the greased plate is consistently more efficient in collecting and holding the dust that falls from the air. For measurement of dustfall over fairly short periods of rain-free weather, the greased plate method has definite advantages over the water-jar method.

ACKNOWLEDGMENT

The authors wish to thank the Dow Chemical Co. for the financial support which made this work possible.

LITERATURE CITED

(1) Larson, G. P., and coworkers, Air Pollution Control District, Los Angeles County, "Test Procedures and Methods in Air Pollution Control," pp. 53–60.
(2) Mitchell, J. P., *J. Ind. Eng. Chem.*, 6, 454 ff. (1914).
(3) Paxton, R. R., *Rock Products*, 54, No. 2, 114–18; No. 6, 127 ff. (1951).
(4) Pond, R. L., "Development of Coated Plate Method for the Measurement of Dustfall Rate" M.S. thesis in chemical engineering, Stanford University, 1954.
(5) Youden, W. J. "Statistical Methods for Chemists," pp. 20–3, Wiley, New York, 1951.

RECEIVED for review June 28, 1954. Accepted February 16, 1955.

END OF SYMPOSIUM

Other papers in this symposium are published in the May issue of *Industrial and Engineering Chemistry*

Principles of Precision Colorimetry

A General Approach to Photoelectric Spectrophotometry

CHARLES N. REILLEY and CRAYTON M. CRAWFORD
University of North Carolina, Chapel Hill, N. C.

This paper is an inquiry into the effect of slit width, sensitivity, and dark-current knob settings on spectrophotometric precision, with a view toward the development of improved high-precision methods. An expression for the relative error is derived, and the conditions for its minimization are discussed under assumptions sufficiently general both to include and extend previous techniques. Four methods are distinguished, of which two are new. One of the new methods gives the best precision obtainable, using two reference solutions and other conditions selected to make this statement true. The selection procedure is described. The other new method is applicable to trace analysis and represents a compromise when solutions sufficiently concentrated to permit optimum conditions are not available. Both methods promise in their respective applications substantial improvement in precision over former methods at small extra cost in time and effort.

THE analyst using a photoelectric spectrophotometer has several instrumental controls at his disposal, which he may adjust as his needs dictate. It has long been recognized that best results are obtained with the wave-length knob set at an absorption maximum, assuming no interferences. The slit-width control setting is not critical so long as the light is sufficiently monochromatic that no appreciable apparent negative deviation from Beer's law occurs. The sensitivity and dark-current knobs are primarily adjustments to fit the amplified photocell output to the limits of the scale on which this output is to be measured. The present paper is an inquiry into what effect the slit-width, sensitivity, and dark-current settings have upon the precision of concentration measurement under various modes of operation. As a result of this inquiry new precision methods are developed and the spheres of usefulness of both new and old methods are made clearer.

CLASSIFICATION OF METHODS

Photoelectric methods of spectrophotometry usually have in common the adjustment of the instrument to read first 0 and then 100, under specified conditions. According to the choice of these conditions, four methods of operation may be distinguished:

Previously Described Methods. I. "ORDINARY" METHOD. The instrument is set to read 0 with the photocell in darkness and to read 100 when exposed to light which has passed through pure solvent.

II. "TRANSMITTANCE-RATIO" METHOD. The instrument is set to read 0 with the photocell in darkness and to read 100 when exposed to light which has passed through a reference solution somewhat more dilute than the sample.

Proposed Methods. III. "TRACE ANALYSIS" METHOD. The instrument is set to read 100 when exposed to light which has passed through pure solvent and to read 0 when exposed to light which has passed through a reference solution somewhat more concentrated than the sample.

IV. "GENERAL" METHOD. This is potentially the most precise of all. The instrument is set for both 0 and 100 using reference solutions.

INSTRUMENT READING–CONCENTRATION RELATIONSHIP

For any of these four methods an experimental calibration curve of reading *vs.* concentration must be determined, because a given system may not conform to theory, and in any case the constants involved are not otherwise known. The theoretical curves are of interest, however, as predictions of, and first approximations to, actual behavior. The relation to be expected between instrument reading and concentration of absorbent when both the 0 reference and the 100 reference are allowed to vary, which is the relation for Method IV, will therefore be derived and will of course include as special cases the well-known relations of Methods I and II and also that of the proposed Method III.

Several ordinarily well justified assumptions are necessary. First it is assumed that the instrument reading is a linear function of the light issuing from the sample. This means that one may write an equation of the form:

$$R = kI + k' = k_\lambda k_c I + k' \quad (1)$$

where R is the instrument dial reading, k is the sensitivity, I

is the amount of light issuing from the sample, and k' is set by the dark-current control or its equivalent.

For those instruments which depend upon reading the actual photocell or photocell-plus-amplifier output, the sensitivity k can be considered as the product of two factors, k_λ and k_c. The first of these, k_λ, is the "photocell" sensitivity (having units such as amperes per photon or volts per photon) and carries the subscript λ to indicate its dependence on wave length. On the other hand, k_c, which is the "circuit" sensitivity (having units such as scale divisions per ampere or scale divisions per volt), is independent of wave length.

On direct-reading instruments, k_c is the quantity controlled by the sensitivity knob and represents the amplification factor of the amplifier.

On null-point instruments, the reading depends on opposing the photocell current or voltage with an auxiliary current or voltage read on an arbitrary scale. The amplifier is usually used only to provide a sensitive null detector. On these instruments the sensitivity knob determines the buck-out voltage or current per scale division and therefore sets k_c, but this does not represent the amplification factor of the amplifier, since the signal is not ordinarily amplified.

On instruments which employ photocells solely to determine whether two light beams are of equal strength, the reading is made by varying the amount of photocell light in one beam with iris diaphragms, by rotating polarizing prisms, or the like, so that providing a k_c control is not ordinarily convenient.

Some control over k_λ can be exerted if needed by changing phototubes and phototube load resistors.

Using the sensitivity and dark current controls, the following "boundary" conditions are established.

$$R = 100 \quad \text{when} \quad I = I_1 \tag{2a}$$

$$R = 0 \quad \text{when} \quad I = I_2 \tag{2b}$$

On instruments not having k controls, the boundary conditions may still be established by using the slit-width controls to change the amount of light incident upon the reference absorbers. This can also be done, of course, when the instrument does have a k control.

These conditions put into Equation 1 permit k and k' to be found in terms of I_1 and I_2,

$$k = \frac{100}{I_1 - I_2} \tag{3}$$

$$k' = \frac{-100\, I_2}{I_1 - I_2} \tag{4}$$

and thus eliminated, to obtain:

$$R = 100\,\frac{(I - I_2)}{(I_1 - I_2)} \tag{5}$$

where I is the amount of light passing out of any desired test solution, and I_1 and I_2 are the amounts of exit light from chosen references.

The reference quantities I_1 and I_2 can be chosen in only four ways, which are illustrated in Figure 1 and correspond in numbering to the four methods already listed.

The second assumption is that the expression:

$$I = I_0 10^{-a_s bC} = I_0 e^{-2.303\, a_s bC} = I_0 e^{-uC} \tag{6}$$

represents the relation between I and C, or is in error only by a multiplicative or additive constant. In this equation I_0 is the amount of incident light, a_s is the molar absorbancy index, b is the optical path length, C is the concentration of the supposed absorber, and u is introduced as a convenient abbreviation for the frequently occurring quantity, $2.303\, a_s b$.

Three equations of the form of Equation 6 may be written, involving I, I_1, I_2, and C, C_1, and C_2, respectively. When these are substituted into Equation 5 and the common factor I_0 cancelled, one gets:

$$R = 100\,\frac{(e^{-uC} - e^{-uC_2})}{(e^{-uC_1} - e^{-uC_2})} \tag{7}$$

In cancelling I_0 a third assumption has been introduced—namely, that the intensity of the light source is constant or the variations are compensated. The form of Equation 7 shows why a multiplicative or additive constant error in Equation 6 would not affect the relation between R and C, and this fact makes it unimportant whether transmittancies or transmittances are used for e^{-uC} in this equation as long as one is consistent in such use.

In practice, the concentration, C, is found from the dial reading R, so that Equation 7 is solved explicitly for C to obtain:

$$C = -\frac{1}{u} \ln \left[\frac{R}{100}(e^{-uC_1} - e^{-uC_2}) + e^{-uC_2}\right] \tag{8}$$

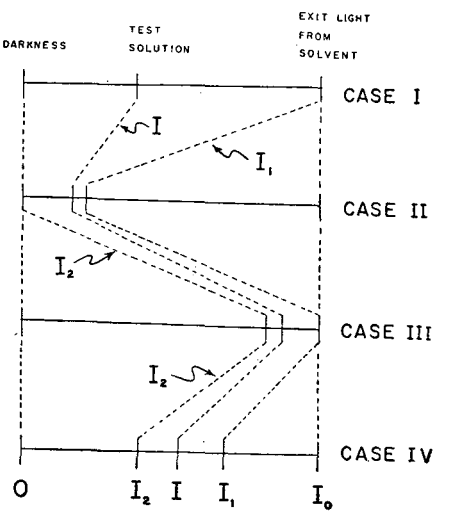


Figure 1. Comparison of spectrophotometric procedures

Case I. "Ordinary" method, darkness–pure solvent references
Case II. "Transmittance-ratio" method, darkness-solution references
Case III. Proposed "trace analysis" method, solution-pure solvent references
Case IV. Proposed "ultimate precision" method, solution-solution references

This equation reduces to more familiar forms in special cases. Each exponential term is the transmittancy of the particular reference denoted by the subscript on C. For the ordinary darkness-solvent references of Method I, the transmittancy of reference 1 is unity and of reference 2 is zero. Hence, for Method I,

$$C = -\frac{1}{u} \ln \frac{R}{100} \tag{9}$$

For Method II the transmittancy of reference 1 is not unity and so does not disappear as such, but the transmittancy of reference 2 (darkness) is still zero. Then for Method II,

$$C = -\frac{1}{u} \ln \left[\frac{R}{100} e^{-uC_1}\right]$$

$$= C_1 - \frac{1}{u} \ln \frac{R}{100} \tag{10}$$

For completeness, the relation for Method III is given:

$$C = -\frac{1}{u} \ln \left[\frac{R}{100}(1 - e^{-uC_2}) + e^{-uC_2}\right] \tag{11}$$

Equation 8 is of course the relation for the general case of Method IV.

ADJUSTMENT OF CONTROLS

Returning to Equation 1, it is seen that the equation defines a family of curves, a typical member of which is shown in Figure 2.

There will always be a practical maximum length to which it is feasible to make a spectrophotometer dial, and it is common practice to divide whatever length is chosen into 100 equal parts, as indicated in Figure 2.

The particular member of the family of curves which is needed may be chosen with the instrumental controls as follows: For a given wave-length setting, the sensitivity knob determines the slope, k, of the curve. The dark-current knob sets the intercept, or k'. Finally, the slit-width control determines the maximum light available and hence the maximum horizontal extension of the curve.

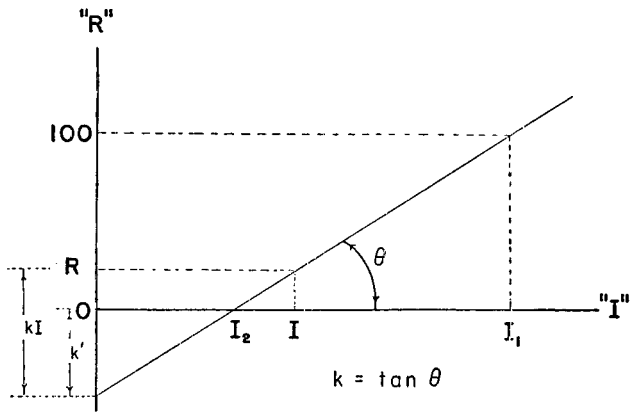


Figure 2. Generalized spectrophotometer working curve

By inserting the value for k given in Equation 3 into Equation 4, and substituting the result into Equation 1, one obtains

$$R = kI - kI_2 = k(I - I_2) \qquad (12)$$

Then applying the condition of Equation 2a, as well as Equation 6,

$$k(I_1 - I_2) = kI_0(T_{s1} - T_{s2}) = 100 = \text{constant} \qquad (13)$$

This is true for all four methods, and in particular for III and IV. For I and II this simplifies to

$$kI_1 = kI_0\, T_{s1} = 100 = \text{constant} \qquad (14)$$

These results are useful in predicting the behavior of spectrophotometers (see the section on selecting the Method III reference solution).

The four methods listed earlier may be redescribed in terms of these ideas.

Methods I and II. (*a*) With I equal to zero, k' is made equal to zero with the dark-current control (see Figure 3). (*b*) With an absorber (solvent for Method I, standard solution for Method II) the product kI is adjusted to $kI_1 = 100$ by any suitable combination of values of k and I, which means any suitable positions of the sensitivity knob and slit-width knob.

The adjustment *b* may be made in two ways (or, of course, any combination of these ways). With a random setting of sensitivity and slit-width controls, the working curve may be, for example, OA in Figure 3. Since this curve does not use the whole instrument dial, stretching to the line marked $R = 100$ is desirable. A stepless sensitivity control can be used to rotate the curve about 0 until its end point meets $R = 100$ at B. (The length of the curve is not fixed by I; it is the horizontal extension of the curve only which is fixed.) If the sensitivity can only be adjusted in steps, the curve will in general fall short of B or overshoot, so that the second and following way of making this adjustment must be used. From the initial setting of the controls to give curve OA, an increase in slit width will extrapolate the curve to C, so that $R = 100$ is achieved at an amount of light of I_1'. The working curve is then OC.

Methods III and IV. These methods have in common the fact that k' is not made equal to zero when I is zero, but is adjusted so that the instrument reads zero with nonzero light set by a standard absorber. This is done on instruments which read photocell output by supplying a "buck" current or voltage with the dark-current control (making a misnomer) or with its equivalent such as an opposing photocell, using an iris diaphragm for adjustment.

What is the best combination of values of k and I_0? No unique answer can be given, but the following criteria apply:

Upper Limit on I. The slit width should be small enough for the molar absorbancy index to be fairly constant over the wavelength passed by the slit. Failure to do this will result in negative apparent deviations from Beer's law with resulting loss in precision.

If the maximum light is too high, deviations from linearity of photocell response and nonreproducibility of reading through fatigue effects may be observed. Only the nonreproducibility is really important, since only a short section of the response curve is ever needed.

Lower Limit on I. I must be large enough so that excessive amplification of the photosignal will not be necessary—that is, I must be large enough so that values of k large enough to cause instrumental instability will not be required. The maximum permissible amplification is fixed by the signal-to-noise ratio, which may be increased if necessary by such devices as refrigeration of critical parts, negative feedback, and electronic stabilization of the power input to the light source. This is, however, really a design problem, because there is no point in providing positions of the sensitivity knob which have too much instability.

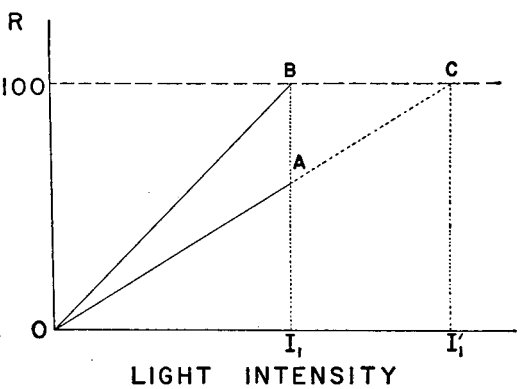


Figure 3. Adjustment of boundary conditions, Methods I and II

Stray light may become important in some cases. Large values of I should tend to decrease this error percentagewise if the stray light arises from an independent external source.

CURVE OF LOG R *vs.* C

Comparison of Equation 8 with the various forms to which it reduces under the different methods is of interest. Equations 9 and 10 show that a plot of log R *vs.* C yields a straight line for both Methods I and II. Since the permissible range of R is 0 to 100, the range of log R is from minus infinity to +2, regardless of the method used. For Method I, this plot will look like curve I of Figure 4, and for Method II like curve II. With these two methods the concentration is allowed to go to infinity, since in principle only an infinitely concentrated solution would absorb all the light, giving a darkness reference. For Methods III and IV, however, the instrument is set to read R = zero

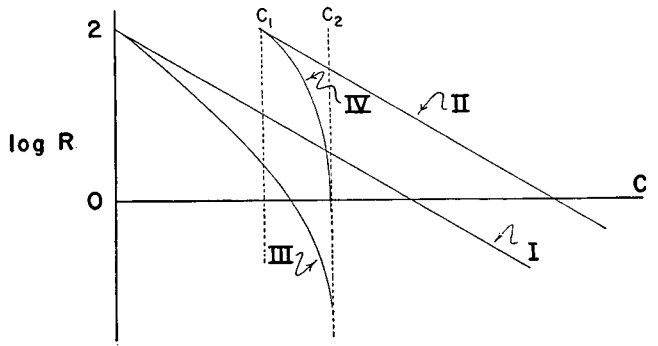


Figure 4. Idealized calibration curves, Methods I, II, III, and IV

when $C = C_2 \neq \infty$, so that log R becomes infinite for finite C. Thus it is geometrically impossible for this curve to be linear when the darkness reference is abandoned, since the curve is asymptotic to the line $C = C_2$, as shown in curves III and IV, Figure 4, drawn for their correspondingly numbered methods.

INCREASED PRECISION DUE TO POSITIVE DEVIATION

The direction of curvature is seen to be such as to give a positive apparent deviation from the curve I, or Beer's law, curve. The importance of this is shown by the following quotation from Hiskey (*3*): "Only in rare cases will it be possible to arrange the experimental conditions to achieve a high degree of positive deviation. When this can be done, however, a large gain in precision may be expected." By abandoning a darkness reference these formerly "rare cases" may be made commonplace.

To see why positive deviation gives greater precision, consider Figure 5. Subject to careful interpretation of the term "uncertainty," this figure shows that a given uncertainty A in log R will produce the uncertainty B in concentration anywhere on the Beer's law curve, but along any curve having a greater slope —i.e., along any curve showing positive deviation—the uncertainty C in concentration will be relatively less than B and will decrease as the deviation increases.

Returning to Figure 4, with a given choice of references, C_1 and C_2, it is seen that the slopes, and hence the precisions, of the methods bear the following relations to each other: IV>III>I and IV>II>I. The relative merits of II and III cannot easily be determined from this graph and will be discussed later. A word of caution is in order regarding the interpretation of Figure 5. The figure shows that a decrease in actual concentration error occurs with increased slope, but in order for decreased relative error to occur the decrease in actual error must occur for the same concentration. A moment's inspection shows that this is true for the curves drawn for the pair I and III and for the pair II and IV in Figure 4. Since curves I and II have the same slope, the same actual concentration error occurs with both, but inasmuch as curve II is at a much larger concentration, the relative error decreases.

APPLICATION TO TRACE ANALYSIS

Previously, positive deviation has been found mainly when the absorbing material is subject to an equilibrium such that at high dilutions the concentration of the colored species is decreased more than the dilution would account for. In such cases one is unable to take advantage of all the precision which the magnitude of the molar absorbancy offers, at least until high concentrations are reached; and this fact limits the usefulness of the system for trace analysis. Other less frequently encountered causes of positive deviation are of little value for similar reasons.

Neither can trace analysis take advantage of the increased precision associated with the transmittance-ratio Method II, for trace analysis necessarily measures extremely dilute solutions with which the use of any except a solvent reference for the 100 setting becomes unprofitable and troublesome.

Method III for trace analysis, here proposed, suffers from no such limitations. It uses positive deviation due to abandonment of a darkness zero, in a way to be described, to increase greatly the precision of trace analysis measurement.

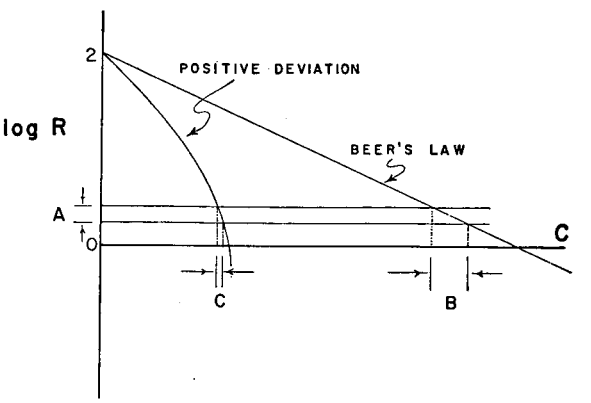


Figure 5. Decrease of error with positive deviation

On the other hand, with major constituents the analyst may select any absorbancy at all for measurement, as it is easy to dilute concentrated solutions. High absorbancy samples, unless diluted, make the use of any but a darkness zero impractical, and for this case Method II provides greater precision than Method I. However, one may inquire whether the use of less concentrated solutions and two solution references will not result in still better precision, a possibility inferred from Figure 4. To answer this question a quantitative description of the property demonstrated in Figure 5 is needed.

RELATIVE ERROR IN METHODS

The analyst is more interested in the relative error dC/C than in the absolute error (uncertainty) dC. The relative error may be found by adding together the contributions due to each uncertain quantity in Equation 6:

$$\frac{dC}{C} = \frac{1}{C}\left(\frac{\partial C}{\partial R}\right)_{C_1,\,C_2,\,u} dR + \frac{1}{C}\left(\frac{\partial C}{\partial C_1}\right)_{C_2,\,u,\,R} dC_1 + \frac{1}{C}\left(\frac{\partial C}{\partial C_2}\right)_{C_1,\,u,\,R} dC_2 + \frac{1}{C}\left(\frac{\partial C}{\partial u}\right)_{C_1,\,C_2,\,R} du \quad (15)$$

Carrying out the indicated operations, one finds:

$$\frac{dC}{C} = \frac{1}{uCe^{-uC}}\left[\left(\frac{e^{-uC_1} - e^{-uC_2}}{100}\right) dR - \frac{uR}{100} e^{-uC_1} dC_1 - u\left(1 - \frac{R}{100}\right) e^{-uC_2} dC_2 + \left(C_2\left\{\frac{R}{100} - 1\right\} e^{-uC_2} - \frac{R}{100} C_1 e^{-uC_1} + Ce^{-uC}\right) du\right] \quad (16)$$

MINIMIZATION OF ERROR

In principle, Equation 16 permits calculation of the values which the parameters should have for minimum relative error. Unfortunately, the calculation is not independent of the uncertainties, which are neither readily obtained nor constant with varying instruments and absorbers. Furthermore, even if

the uncertainties were known, the calculation of the optimum parameter values presents a task to be undertaken only by a machine computer because of the enormous amount of work involved, which must be repeated each time a change of instrument or absorber changed the uncertainties.

The best practical way out of these difficulties is as follows. The dependence of the coefficient of du on u is such that this contribution is minimized when u is large, which can be achieved by selecting absorbers having a large molar absorbancy and by using long optical paths. The coefficients of dC_1 and dC_2 are not subject to manipulation, but these uncertainties are made small by careful attention to all volumetric operations, as recommended by Young and Hiskey (*7*). Under these conditions, one hopes that the biggest error is instrumental, and this error is minimized by selecting R, C_1, and C_2 to minimize the "error coefficient," which except for a constant factor is the coefficient of dR:

$$\frac{e^{-uC_1} - e^{-uC_2}}{T_s \ln T_s}$$

where T_s is the transmittancy of the sample, a quantity independent of method.

The preceding statement requires amplification. The usual practice is to minimize $\frac{dC}{C} \Big/ dR$, and the description in this paper up to this point has been in terms of this idea. However, it is difficult to see how minimizing anything but the relative error itself—i.e., dC/C—will minimize the relative error. The usual practice can be justified only by assuming dR to be constant—i.e., independent of the choices of u, C_1, C_2, and R.

Gridgeman (*2*) has pointed out that the kind of error analysis involved in setting up methods through investigations of $\frac{dC}{C} \Big/ dR$ is dependent on the geometrical properties of the log R *vs.* C curve (demonstrated in Figure 5) and is unrelated to any theory of the source of the error. By this means a diminution in the relative error in concentration due to unit error in determination of the instrumental reading may be had; whether this is to be translated into any real diminution in relative concentration error depends on how many units of instrumental reading error must be accepted simultaneously.

CONTRIBUTIONS TO READING ERROR

The error, dR, in reading a spectrophotometer dial is the sum of several contributions. Some one contribution is commonly much more important than the rest, and not all possible contributions will necessarily occur in any one spectrophotometer. At least the following contributions can be present when:

A. The pointer is in the correct place with respect to the ends of the dial.

1. Error made because the calibration marks are misplaced with respect to the ends of the dial, so that wrong judgment of pointer position is made.
2. Error made because the eye cannot estimate the position of the pointer with respect to the calibration marks more accurately than about 0.1 of the least count.

B. The pointer is at the wrong place with respect to the ends of the dial.

1. Wrong because the desired signal is not able to move it to the correct place.
2. Wrong because no desired signal is present to cause it to move to the correct place.
3. Wrong because undesired signals move it away from the correct place.

The dR_{A1} errors are not necessarily constant, but if large are easily corrected for and if small are unimportant.

The dR_{A2} errors are constant with a given observer on a linear scale. With some instruments, particularly those which use the photocell only to indicate the equality of two light beams, and which use some device such as the rotation of polarizing prisms to cut down on beam by a known amount to match the other, the calibration of the R scale necessary to establish the relation of Equation 1 is rarely linear and dR_{A2} may vary with R. An example of this and a discussion of minimizing relative concentration error for Method I in the event that this error dominates dR may be found in a chapter by Stearns (*6*). A similar minimization may be used with Methods II, III, and IV.

The dR_{B1} errors arise through static friction which must be overcome by the signal before movement is possible. These errors are thus inversely proportional to the sensitivity, k; by working at constant k they become constant. They are usually very small.

The dR_{B2} errors can arise through play in a gear system, or through lack of sensitiveness in a null-point detector. An example of the latter is the Beckman DU, which at high sensitivity lacks a sharp null. In fact, over the range in which this error is the dominant contribution to dR, the error is proportional to the sensitivity k, and may thus be minimized by working at a low value of k. For constant k the error is constant.

Gear system play can usually be eliminated as an error by uniformly approaching a setting from the same direction, or by subtracting the amount of the play from all readings made from the opposite direction.

The dR_{B3} errors are the most serious and the most difficult to treat theoretically. If the unwanted signals are introduced at the input to the amplifier, and thus amplified, the dR_{B3} error produced is proportional to the amplifier sensitivity k_a, and hence to k. If introduced within the amplifier, the unwanted signals will be a less sensitive function of k, and when the unwanted signals are introduced into the amplifier output they are independent of k.

LIMITATIONS DUE TO LIGHT FLUCTUATIONS

The most common source of unwanted signals is a fluctuating light source. Here the unwanted signal dI is introduced at the input to the amplifier, so that the error dR_{B3} produced is proportional to the sensitivity, k. For constant sensitivity the error is constant. But this means that Methods II, III, and IV can be applied only by increasing I_0. If this is done with the slit width control, the fluctuation increases, since dI_0/I_0 = constant. The error dR_{B3} is then proportional to I_0, so that the application of Methods II, III, or IV necessarily entails a greater dR_{B3}.

The argument can be made more valuable by considering the whole instrumental error due to light fluctuations. This is:

$$\frac{1}{C}\left(\frac{\partial C}{\partial R}\right)_{u,\,c_1,\,c_2} dR = \frac{(e^{-uC_1} - e^{-uC_2})}{100\ T_s \ln T_s}\, dR$$

By Equation 6, this may be written:

$$\frac{1}{C}\left(\frac{\partial C}{\partial R}\right)_{u,\,c_1,\,c_2} dR = \frac{1}{I_0}\frac{(I_1 - I_2)}{100\ T_s \ln T_s}\, dR \qquad (17)$$

Substituting $I = T_s I_0$, where T_s is the transmittancy of the sample, into Equation 1 and differentiating yields:

$$dR = kT_s\, dI_0 \qquad (18)$$

But

$$\frac{dI_0}{I_0} = k'', \text{ or } dI_0 = k''I_0. \qquad (19)$$

Also, $k = \frac{100}{I_1 - I_2}$ (from Equation 3)

Hence, substituting Equations 3 and 19 into Equation 18 and the result into Equation 17,

$$\frac{1}{C}\left(\frac{\partial C}{\partial R}\right)_{u,\,c_1,\,c_2} dR = \frac{1}{I_0}\frac{(I_1 - I_2)}{100\ T_s \ln T_s} \times \frac{100}{(I_1 - I_2)} \times T_s k'' I_0 = \frac{k''}{\ln T_s} \qquad (20)$$

In Equation 20 the entire right-hand side is composed of con-

stants depending only on the sample. The left-hand side is the relative concentration error. Yet no choice of I_1 or I_2 has been made in the derivation, so that the instrumental precision must be precisely the same for all four methods. Because of the $\ln T_s = -uC$ denominator a highly concentrated sample is advantageous for precise measurement, but no advantage will result from using Method II despite this method's normal advantages in concentrated samples.

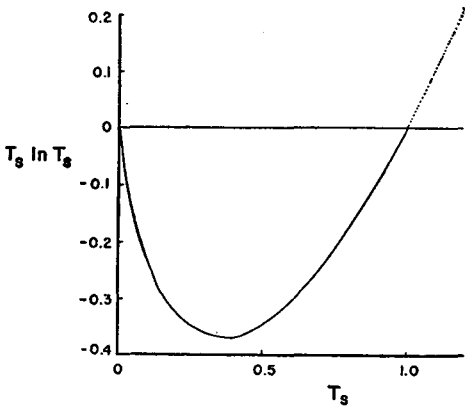


Figure 6. Behavior of error coefficient denominator

A possible objection to this derivation arises from the fact that Equation 6 from which the error coefficient was found was derived subject to the assumption of constant I_0. If the derivation is repeated with this restriction removed, and no compensation provided, Equation 17 still results, and the conclusion regarding the purely instrumental error is still valid. In this case, however, another term must be added to Equation 15:

$$\frac{1}{C}\left(\frac{\partial C}{\partial I_0}\right)_{u,\,C_1,\,C_2,\,R} dI_0 = -\frac{dI_0}{uI_0C} \qquad (21)$$

But by Equation 19 this is equal to k''/uC, which again is independent of the method used but which gives additional reason for measuring concentrated samples if possible. It is fair to assume that no compensation has been provided because otherwise it would be improbable that light fluctuations are the major contribution to dR.

The conclusions of the preceding paragraphs apply if the dominant term in dR is produced by light fluctuations. The fact that better precision has customarily been reported using Method II must be ascribed to the comparative rarity of light fluctuations as the dominant cause of error in well designed instruments. It is also possible that even if the light fluctuations are dominant at low sample concentrations they may cease to be so with the concentrations normally used with Method II so that some gain results.

Compensation. Compensation of fluctuations of the intensity of the light source is inherent if such fluctuation can be made to affect I, I_1, and I_2 all simultaneously. This follows from the fact that I_0 cancelled in deriving Equation 7. With a single-beam instrument it is difficult to see how this can be accomplished. Since such fluctuation does not affect darkness, with Methods I and II a double beam instrument can be used to make fluctuations affect I and I_1 simultaneously and so provide theoretically perfect compensation. These instruments will not provide perfect compensation for Methods III and IV, though they may be expected to be better in this regard than single beam instruments. An instrument which compensated perfectly in theory with III and IV would undoubtedly be a triple beam instrument.

The degree of compensation provided also can have an important effect on the ease of using Methods III and IV.

MINIMIZING THE ERROR COEFFICIENT

When dR does not depend on the choice of u, C_1, C_2, and R, achieving minimum relative concentration error depends solely on minimizing the error coefficient. This is really a simple matter. The numerator is the difference in transmittancies of the references: To minimize, make this span as short an interval as possible. The denominator $T_s \ln T_s$ is plotted as a function of the sample transmittancy T_s in Figure 6. The function is not defined for negative values of T_s; it begins at the origin, passes through a minimum which differentiation shows occurs at $T_s = 36.8\%$, crosses the abscissa at $T_s = 100\%$, and proceeds to large positive values which are physically meaningless unless the sample emits light of its own making.

Three points are therefore of special interest: the minimum at which the denominator has its largest realizable absolute value and the two zeros where the fraction might be expected to become infinite—i.e., produce infinite error. The denominator zeros are not of great consequence, however, because the numerator is necessarily smaller than the denominator for the best case, and indeed, the limits of the error coefficients as these points are approached are easily shown to be zero for the best selection of references for a given sample.

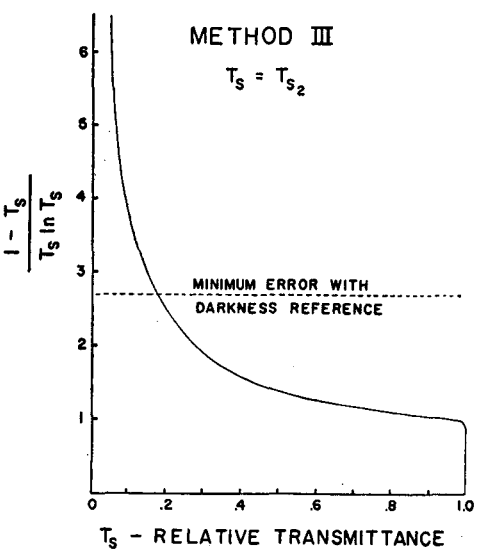


Figure 7. Minimum error, Method III

Reference to Equation 7 shows that the sample transmittancy must lie between the transmittancies of the standards or the reading will be negative or greater than 100. The error coefficient numerator shows that maximum precision will be achieved by Method IV when working with a very small transmittancy span between references, and a span which includes 36.8%; the sample transmittancy must lie within that small span. Good precision will also be obtained as long as this span is small regardless of where on the transmittancy scale the span is located. At a sample transmittancy of 50%, the best application of Method II uses a span between 0 to 50% transmittancy, and the best application of Method III uses a span between 50 to 100% transmittancy, and since the spans are of equal length and the sample transmittancy equal, for this sample the errors of Methods II and III are equal. Below 50% sample transmittancy, Method II is more precise, and above 50% sample transmittancy, Method III is more precise.

Method IV is always more precise than either, but Method II has been shown under favorable circumstances to be capable of precision of the order of one part in 1000 (*1*, *6*), which will be sufficient for most purposes, and Method II has an advantage

in that only one reference need be prepared. The linear calibration curve may be deemed by some to be an advantage of Method II. The ease of dilution of samples and the difficulty of concentrating them, especially when only a small amount is available, seem to assure the usefulness of Method III. Method IV can always substitute for II with a precision advantage gained in the process; neither II nor IV can find practical use in place of III in the case of trace analysis. For solution of somewhat higher concentrations than trace amounts, Method IV becomes profitable. Figure 7 shows the behavior of the relative error for Method III for the best case in which the more concentrated reference has the same concentration as the sample. Figure 7 of Hiskey's paper (*3*) gives the corresponding curve for Method II for the best case when the less concentrated reference has the same concentration as the sample. This is the minimum error by these methods, but in practice neither will be quite as good as this, except in color matching by titration, because of the necessity for running an experimental calibration curve for each change of reference.

USEFUL RANGE OF R

The question then arises as to how far away from the ideal case it is possible to go without undue sacrifice, or, in other words, what is the useful range of R for the various methods. For a given reference transmittancy span, the error coefficient numerator is constant, so that sacrifice of precision comes through decreases in the denominator. Approach to the denominator zeros shown in Figure 6 can for constant references produce infinite error, but since the numerator in such a case is always smaller for Methods II and III than for Method I, a given sample can always be determined with less error by Methods II and III than by Method I. However, there is no point in resorting to the somewhat more complicated Methods II and III unless more than a token gain in precision is achieved. Ultimately the question of the useful range of R will depend on what error can be tolerated, which accounts for the variation in estimates of the useful range of R to be found in the literature. Hiskey (*3*) selects 20 to 65 as the appropriate range for Method I. His Figure 6 indicates that on the low side the knees in the curves continue to come at about 20, but since with decreasing reference transmittancy the curves are lowered, the usable limit for a given error tolerance is lowered below 20 by a small amount.

The upper limit of R for Method II varies much more sharply with reference transmittancy, being 65 for the limiting case of Method I, and increasing with decreasing reference transmittancy. Below a reference transmittancy of 36.8% the limit may be taken as 100.

Figure 8 of the present paper describes the behavior of relative error in concentration with Method III under the assumption of constant dR. As with Method II, one end of the plot has the curve knees at about the same place, namely at about an R value of 65. The other end is more variable, as before; for the limiting case of Method I it is $R = 20$, but with reference transmittancies above 36.8% the limit may be taken as $R = 0$. These results are summarized in Table I.

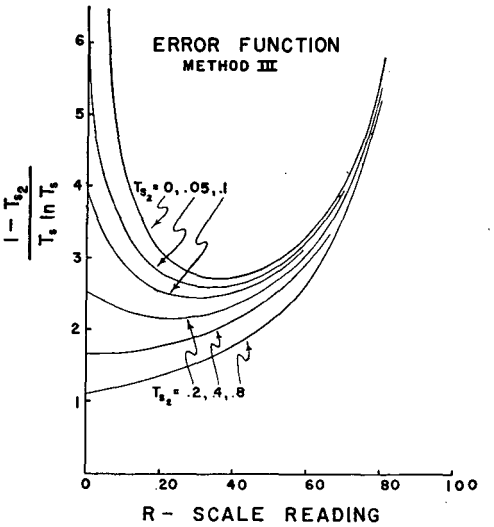


Figure 8. Error of measurement, Method III, *vs.* R

Figure 9 presents for Method III both the absolute and relative concentration error as a function of sample concentration for constant dR. The dotted curves are the actual working curves, while the line labeled $\frac{dC}{C}$, $C = C_2$ presents the relative concentration error for the ideal case. This curve drops to zero in a very short distance along the abscissa, thus illustrating a peculiar property of the method. Usual experience with errors is based on the behavior of the $\frac{1}{C}$ *vs.* C curve illustrated, which becomes large with small C and causes the relative error $\frac{dC}{C}$ to become large. With Method III, however, dC is becoming small faster than C is, so that the relative error decreases for the ideal case. At some limiting dilution, of course, the instrumental error will cease to be the major error and the contribution of the other terms of Equation 15 will keep the actual error from being zero.

Table I. Comparison of Spectrophotometric Methods

Method	Equation	Error Coefficient	Use
I	$C = -\frac{1}{u} \ln \frac{R}{100}$	$\frac{1}{T_s \ln T_s}$	General (special and convenience)
II	$C = C_1 - \frac{1}{u} \ln \frac{R}{100}$	$\frac{T_{s1}}{T_s \ln T_s}$	Precision (high concentration)
III	$C = -\frac{1}{u} \ln \left[\frac{R}{100}(1 - e^{-uC_2}) + e^{-uC_2}\right]$	$\frac{1 - T_{s2}}{T_s \ln T_s}$	Precision (low concentration)
IV	$C = -\frac{1}{u} \ln \left[\frac{R}{100}(e^{-uC_1} - e^{-uC_2}) + e^{-uC_2}\right]$	$\frac{T_{s1} - T_{s2}}{T_s \ln T_s}$	Ultimate precision

	Useful T_s Range of References	Optimum Value of Sample T_s	Useful Range of R (approx.)
I	$T_{s1} = 100 \quad T_{s2} = 0$	36.8	20–65
II	$50 > T_{s1} > T_{s2} = 0$	$\begin{cases} T_{s1} & (T_{s1} < 36.8) \\ 36.8 & (50 > T_{s1} > 36.8) \end{cases}$	20–100
III	$100 = T_{s1} > T_{s2} > 50$	T_{s2} $(T_{s2} > 50)$	0–65
IV	$100 >> T_{s1} > T_{s2} >> 0$	$\begin{cases} T_{s1} & (36.8 > T_{s1} > T_s > T_{s2}) \\ T_{s2} & (T_{s1} > T_s > T_{s2} > 36.8) \\ 36.8 & (T_{s1} > 36.8 > T_{s2}) \end{cases}$	0–100 $(100 >> T_{s1} > T_s > T_{s1} >> 0)$

No significance can be attached to the absolute values of the ordinate in Figure 9, since it is plotted for an arbitrary value of u.

The useful range of R for Method IV will undoubtedly be 0 to 100 whenever the references are both appreciably separated from the end points of zero and 100% transmittancy, and whenever their transmittancies span a reasonably short interval. Which end of the R scale will be most precise will depend on which side of the $T_s \ln T_s$ minimum is being used; if the references are equally spaced on either side of 36.8%, the most precise value of R will be 50.

SELECTING THE METHOD III REFERENCE SOLUTION

The simplest way to select the reference concentration is to use the most concentrated solution expected to be encountered. This selection assumes that the zero and 100 settings can be made for this solution, and such is not always the case. In the latter event, the reference concentration chosen is the least concentrated (highest transmittancy) which can be balanced.

An example of an instrument having a limitation on how dilute a reference can be balanced is the Beckman Model B spectrophotometer. On the highest sensitivity range a more dilute limit is found than with lower sensitivity; the highest sensitivity limit is about 78% transmittancy for the instrument in this laboratory, which is a figure of merit as regards application of Method III. The maximum balanceable reference transmittancy varies with sensitivity, not because the maximum current available from the dark-current control depends on sensitivity, but because when the sensitivity is decreased, the slit width must be increased to satisfy Equation 13. Therefore more photocurrent must be balanced for the same zero-reference transmittancy, and if the dark-current control is already providing its maximum output, this balancing cannot be done.

Direct experimentation to find this least concentration on the Beckman Model B is tedious and time consuming, primarily because setting the zero changes the 100 setting and vice versa. Thus a system of successive approximations to the correct settings must be used for each of many samples until the least concentrated which can be balanced is found.

Fortunately an easier way is available. Select a sample with any transmittancy between 100 and the bounding value—i.e., any sample which cannot be balanced. With the solvent in the light beam, turn the dark-current knob to its extreme position in the direction causing a decreased scale reading. Next open the slit until the instrument reads 100. Insert the sample and record the instrument reading R.

Then if T is the transmittancy of the sample (measured by Method I), it can be shown that the transmittancy of the limiting reference for Method III will be:

$$100\left(1 - \frac{100 - T}{100 - R}\right)$$

For instruments which depend on reading photocell output, the limiting reference transmittancy depends upon the electrical circuit and not upon the optical characteristics of the instrument nor the system measured. Once found by the above process, it need not be reevaluated. A limiting reference transmittancy of 78% means that the conditions of Equation 2 may always be satisfied for Method III using a reference transmittancy less than 78% (now requiring a process of successive approximations to the correct setting for one sample only), but for reference transmittances greater than 78% these conditions cannot be satisfied.

Double-beam instruments are not necessarily subject to this difficulty of using successive approximations to set the references. This is because k' (see Equation 4) is independent of I_0 if I_1 and I_2 are affected simultaneously by any change in I_0, and such occurs with those double-beam instruments which are perfectly compensating for light fluctuations when using Methods I and II.

The successive approximation difficulty can also be circumvented with single-beam instruments if the sensitivity control is stepless, as with the Beckman DU, by making this adjustment of the references with the dark-current control first and then the sensitivity control, at constant slit width. It is desirable to work at high sensitivity to avoid loss of wave-length resolution, but with the model DU there is a conflicting requirement due to the poor detectability of the null point which requires working at low and constant sensitivity.

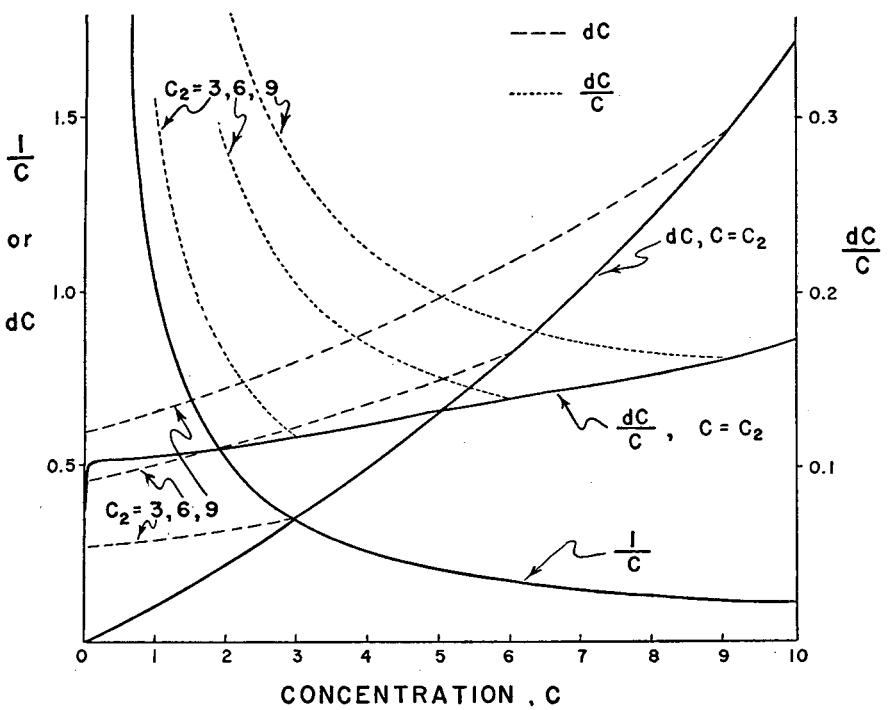


Figure 9. Error of measurement, Method III, *vs.* C

THEORETICAL GAIN IN PRECISION

The theoretical gain in precision with any of these precision methods over Method I may be found very simply by inspection of the error coefficient column of Table I. If one has a sample of given transmittancy and wishes to know how much more precise, for example, Method II is than Method I, he simply divides the reference transmittancy into 100. The simplicity results from the fact that with a given sample transmittancy, the error coefficient denominators are equal for all methods, so that comparisons are made with the numerators alone. It is in this way that the curve of Figure 10 was plotted.

A somewhat surprising and interesting result now becomes evident: that regardless of the value of R (compare section on the useful range of R), the number of times better that these precision methods are than Method I is constant, and fixed by the choice of references. Thus if one can do twice as well for the best case—i.e., $T_s = T_{s2}$—for Method III as Method I, one can still do twice as well as Method I for a

very bad case ($T_s << T_{s2}$), provided the same samples are compared in the two methods. A similar statement can be made about Methods II and IV.

As an example of the use of these error coefficients, consider the limiting reference transmittancy of 78% found for the Beckman Model B for Method III. The numerator of the corresponding error coefficient is 1 − 0.78 = 0.22. This figure divided into the numerator of the error coefficient of Method I (which is 1) gives 1/0.22 = 4.5. This means that under these conditions Method III is 4.5-fold more precise than Method I. The same result may be read directly from Figure 10 as shown by the dotted lines.

OUTLINE OF PROCEDURE FOR METHODS III AND IV

It may prove useful to present an abbreviated outline of the procedure to be followed in an actual analysis.

1. The range of concentration over which it is desired to make measurements is determined, and the extreme points of the range are selected as reference solutions. (If this range is too wide to permit sufficient precision, it may be divided into two or more subranges, each with two terminal references, but too much subdivision should be avoided, since a separate calibration curve must be run for each interval. Also, the extent of subdivision of the subranges may be limited by the adjustability of the instrument.)

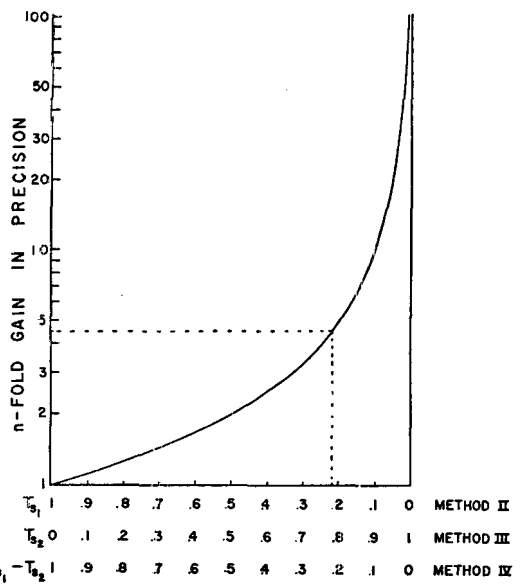


Figure 10. Theoretical gain in precision

T_{s1}. Transmittancy of standard for 100 setting
T_{s2}. Transmittancy of standard for 0 setting

2. A series of solutions of exactly known concentration lying between and including the reference solution concentrations is prepared.

3. The instrument is set on highest sensitivity (unless the chief error is null-point detectability, when the sensitivity is reduced until the chief error is in reading the pointer position) and left in this position.

4. The reference solution of higher concentration is used to set the instrument to read zero while the reference solution of lower concentration is used to set the instrument to read 100, with dark-current and slit-width knobs alone. (This may or may not require a process of successive approximations to the correct setting, depending on the manner of adjustment used and on the type of instrument).

5. Readings are taken on the known solutions of concentration lying between the concentrations of the references, and the data used to plot a calibration curve of concentration *vs.* reading, or of concentration *vs.* the logarithm of the reading, depending on convenience.

6. The unknown(s) are measured, and the concentration is read from the graph.

Table II. Experimental Reflectance Data, Method IV

Standard	Adopted Reflectance, %	Symbol	Reading
	Group I		
2A	1.754	I_1	(100.0)
1A	1.028	I	53.2
0.5A	0.194	I_2	(0.0)
	Group II		
35A	35.47	I_1	(100.0)
20A	23.57	I	51.6
			51.8
10A	10.81	I_2	(0.0)

Table III. Precision of Reflectance Data, Method IV

(Compare with Method I where $dI = \pm 0.1$)

Standard	I, % Measured	I, % Adopted	dI
1A	1.024	1.028	0.004
20A	23.535	23.570	0.035
	23.584		0.014

EXPERIMENTAL

No attempt by the authors has been made to test experimentally the usefulness and validity of the methods described. However, Nimeroff (*4*) carried out some tests, which he has kindly granted permission to describe.

Two groups of three reflectance standards were selected, and a Beckman spectrophotometer (Model DU) was adjusted so that in each group the standard having highest reflectance read 100.0 and the lowest reflectance read 0.0. A reading for the intermediate standard of each group was then taken. The data of Table II were obtained for λ = 440 mμ. From the readings in Group II one can see that there has been no appreciable increase in the uncertainty of reading over usual experience with the "ordinary" method of operation—i.e., ±0.1. When the measured values for R and the adopted values for the high and the low standards of each group were used in Equation 5 and solved for I, the data of Table III were obtained. Since by Method I the reading R would be numerically identical with I and hence I would exhibit the same error—i.e., ±0.1—the error found in this way by Method IV of 0.035 maximum represents at least a threefold improvement, and in Group I a 25-fold improvement.

On the basis of these data Nimeroff concluded that the precision of measuring reflectance is improved by the use of the proposed method. He also mentioned that there were at least two disadvantages of the method:

There is a loss of wave-length resolution because the slits have to be extremely wide to make I_1 read 100. (This is also true of the widely used transmittance-ratio Method II.)

The meter is overloaded when the photocell shutter is closed, which could eventually damage the meter beyond usefulness. Shunting the meter when the shutter is closed could overcome the disadvantage of this feature. When the Model B Beckman spectrophotometer is placed in stand-by position, this is done automatically.

A second experimental test exists in a paper by Ringbom and Österholm (*5*) published after this manuscript was submitted. Their work was with a Lange photometer rather than with a spectrophotometer, and the connection with the present paper is not immediately obvious. No detailed discussion of the correlations between the two papers will be attempted, but analysis shows that their method depends for its validity on the principles described here for Methods III and IV. Again, improved precision was reported.

PRACTICAL CONSIDERATIONS

The range of adjustment of the dark-current knob provided on most instruments will permit the profitable adoption of a non-darkness zero. For many instruments the only modification required to increase this range is replacement of the dark-current

battery with one of higher voltage. It is desirable to be able to compensate completely the photosignal arising from the full light of the source using maximum slit width at the wave length of greatest phototube sensitivity, if this photosignal is to be read, for then the broadest use of Methods III and IV is possible.

CONCLUSIONS

The conclusions of the paper may be summarized as follows: The manner of using the slit-width, sensitivity, and dark-current controls does have an effect on the relative error of measurement of concentration unless the limiting precision of reading the dial is due to light source fluctuations. In the latter event the usual method of using a spectrophotometer—i.e., using darkness and solvent to set the zero and 100 dial positions respectively—is recommended on account of its simplicity, as the precisions of all methods are then nearly or exactly equal.

If the limiting precision of reading the dial is not due to light fluctuations, resort to other methods is determined by the need for precision—that is, the allowable error. The darkness-solvent references of Method I are convenient, rapid, and easily explained to untrained personnel, so that unless a real need for additional precision exists Method I is preferable.

When very precise work is required, the type of work determines the method used. For very concentrated samples, Method II may be used, with the advantage that only one reference solution is needed. Still better precision may be had by diluting the sample to about 36.8% transmittancy and using Method IV, but an additional reference solution is required. For trace analysis on very dilute solutions Method III is used, again requiring only one reference solution. If the precision of reading the dial is limited by the insensitivity of a null-point detector, the method should be used with low and constant sensitivity.

It must be kept in mind that the over-all error will not be better than the care with which volumetric, chemical, and other non-instrumental processes are carried out, irrespective of the method used.

When the new methods here proposed are used properly, a large increase in precision may be expected.

ACKNOWLEDGMENT

This research was supported in part by the United States Air Force, through the Office of Scientific Research of the Air Research and Development Command.

LITERATURE CITED

(1) Bastian, R., ANAL. CHEM., **21**, 972 (1949).
(2) Gridgeman, N. T., *Ibid.*, **24**, 445 (1952).
(3) Hiskey, C. F., *Ibid.*, **21**, 1440 (1949).
(4) Nimeroff, I., National Bureau of Standards, Washington, D. C., private communication.
(5) Ringbom, A., and Österholm, K., ANAL. CHEM., **25**, 1798 (1953).
(6) Stearns, E. I., in "Analytical Absorption Spectroscopy," M. G. Mellon, ed., Chap. 7, p. 338, Wiley, New York, 1950.
(7) Young, I. G., and Hiskey, C. F., ANAL. CHEM., **23**, 506 (1951).

RECEIVED for review November 9, 1953. Accepted December 28, 1954.

Automatic Photometric Titrations of Calcium and Magnesium in Carbonate Rocks

LEONARD SHAPIRO and W. W. BRANNOCK

U. S. Geological Survey, Agricultural Research Center, Beltsville, Md.

Rapid nonsubjective methods have been developed for the determination of calcium and magnesium in carbonate rocks. From a single solution of the sample, calcium is titrated directly, and magnesium is titrated after a rapid removal of R_2O_3 and precipitation of calcium as the tungstate. A concentrated and a dilute solution of disodium ethylenediamine tetraacetate are used as titrants. The concentrated solution is added almost to the end point, then the weak solution is added in an automatic titrator to determine the end point precisely.

THE determination of calcium and magnesium in carbonate rocks by titration with the sodium salt of ethylenediamine tetraacetate (Versene) has been reported by several workers (*1*, *3*). These procedures are more rapid than the conventional gravimetric methods, but it has been difficult for many workers to determine the exact end point in titrations by the usual visual means. Such procedures are suitable for work where high accuracy is not required and where results less accurate than those attained gravimetrically are acceptable. Higher accuracy can be attained by observing the end point as Versene is added to a solution placed in a photometer. This type of titration has been described recently (*4*), but procedures of this kind, which involve incremental additions of Versene solution, are not suitable for routine analyses of large numbers of samples.

The U. S. Geological Survey has had a need for rapid methods of analysis of limestones and dolomites. As a first approach a method was devised to determine calcium and magnesium in which the titrations were done by allowing a solution of Versene to flow slowly, at a constant rate, into a beaker placed between the light source and the photocell of a commercially available spectrophotometer. As the Versene reacted with the calcium or magnesium in the beaker the changing absorption of light was recorded continuously using a pen-and-ink recorder. It was found possible by this arrangement to achieve results within 1 to 2% of the amount present. These titrations required as much as 10 minutes for each titration. In place of the commercial spectrophotometer a titrator was designed for this specific application (Figure 2). This instrument is similar to a titrator described by Barredo and Taylor (*2*).

DISCUSSION OF PROCEDURES

The procedures now used in this laboratory for carbonate rocks are more accurate and more rapid than those obtained by the authors' first approach. In place of the titration of an entire aliquot by the titrator, the procedure is as follows.

A portion of the contents of the titration beaker containing the sample is temporarily withdrawn. The remaining solution is then titrated rapidly with a buret containing a relatively strong solution of Versene, to a point where the color change of the indicator has definitely occurred as observed visually. The volume delivered by the buret is read carefully. The portion of the sample previously removed is now replaced in the titration beaker, bringing the indicator back to its previous color. The titration beaker is now placed into the automatic titrator, and the

small part of the calcium or magnesium not yet reacted is titrated with more dilute Versene. The sum of the results of the two titrations provides an accurate result for the sample.

Both constituents are determined in aliquots from a single solution of the sample. Calcium is titrated directly without separations. Magnesium is determined after rapid separation of the R_2O_3 group with ammonium hydroxide and precipitation of calcium as the tungstate. If R_2O_3 is not removed, results may be low by several tenths of a per cent. Calcium should be separated prior to the determination of magnesium, especially where the calcium-magnesium ratio is high, to avoid the errors arising in difference determinations. The use of tungstate as a precipitant for calcium is more satisfactory than oxalate, which is commonly used.

INSTRUMENTATION

The titration assembly (Figure 1) consists of a Mariotte bottle, a titrator, and a recorder.

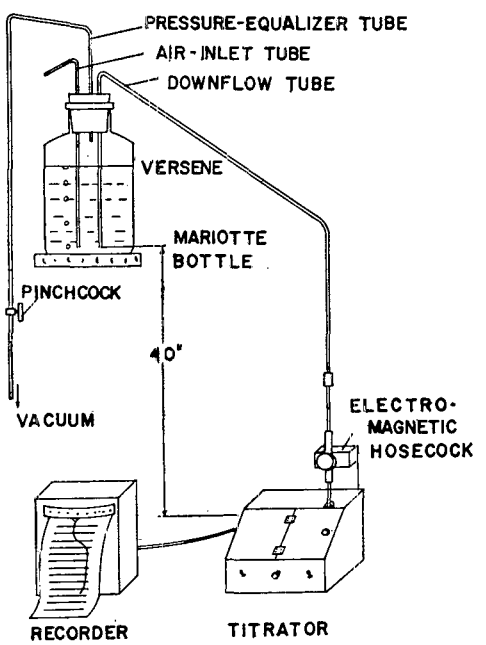


Figure 1. Titration assembly

Mariotte Bottle. A Mariotte bottle provides a simple means for delivery of Versene solution to a titration beaker at a constant flow rate. A 20-liter bottle is mounted on a shelf or support about 4 feet above the work bench. It is fitted with a rubber stopper through which pass three glass tubes. The air-inlet tube starts at a point near the bottom of the bottle, goes through the stopper, and opens into the air. The pressure-equalizer tube starts in the air space above the liquid, goes through the stopper, and is connected to a vacuum line. A rubber section with a pinchcock is placed somewhere along this tube, so that vacuum may be conveniently applied or shut off. The downflow tube starts close to the bottom of the bottle and passes through the stopper and down to the electromagnetic hosecock, which is part of the titrator. A few inches below the electromagnetic hosecock a capillary tube extends that has a tip so constricted that the flow rate is maintained at 4 to 5 ml. per minute.

By this arrangement when liquid is flowing through the downflow tube the hydrostatic head is from the bottom of the air-inlet tube (essentially the bottom of the bottle) to the top of the liquid in the beaker, which is in the titrator. This maintains a constant head as the liquid in the bottle is used up. To keep this hydrostatic head correct the air pressure within the bottle must be such that as liquid is removed, air is entering through the air inlet and bubbling through the Versene solution. As the temperature in the room changes from day to day, it may be necessary to reduce the air pressure above the liquid before the titrator is used. Vacuum is applied until air starts to bubble through the Versene solution, and then the vacuum is turned off. The rubber stopper must, of course, provide an airtight seal to maintain the vacuum.

Titrator. In Figure 1 the titrator is ready to use; in Figure 2 it is shown with the front panels removed. The wiring diagram is given in Figure 3. There are four simple circuits: the stirrer circuit, *a*, the titrator circuit, *b*, the light circuit, *c*, and the photocell circuit, *d*. The stirrer circuit consists merely of a magnetic stirrer with an on-off switch that is connected directly to the line current. The titrator circuit is a single-pole double-throw toggle switch connected in such a way that line current is allowed to go either to the electromagnetic hosecock or, when thrown to the opposite position, to the chart drive of the recorder. By these means coordination is achieved between the opening of the hosecock, which starts the flow of Versene, and the start of movement of the chart. The light circuit is fed from the line, through a voltage stabilizer, to the primary of a 6- to 8-volt transformer. The secondary of the transformer is in series with a 50-cp. automobile lamp bulb and a 50-watt–5-ohm rheostat.

The photocell circuit is a series circuit involving a barrier-layer selenium photocell, a 1.5-volt dry-cell battery, and the galvanometer of the recorder. In this application, advantage is taken of the decrease in resistance of the photocell as the light intensity is increased rather than the output of the photocell itself. The polarity of the units must be correct, as shown in the diagram, or the circuit will not function. A few minutes of experimenting at this point will yield the proper arrangement. A carrier for two 2 × 2 inch filters is mounted in front of the photocell. An orange filter (Corning 3480) is used in titrating magnesium, and this plus a green filter (Corning 4015) is used in titrating calcium.

Recorder. A pen-and-ink recorder that provides full scale deflection for 1 ma. is used. It should have an internal resistance not exceeding 2000 ohms. The chart drive is geared to a feed of 3 inches per minute.

REAGENTS AND APPARATUS

Dilute Versene, 20 grams of disodium ethylenediamine tetraacetate dissolved in 20 liters of water. Place in the Mariotte bottle.

Concentrated Versene, 7.0 grams of disodium ethylenediamine tetraacetate dissolved in 4 liters of water.

Hydrochloric acid, 1 + 3 in water.

Sodium hydroxide, 15% in water.

Buffer solution, 66 grams of ammonium chloride per liter of 1 + 1 ammonium hydroxide.

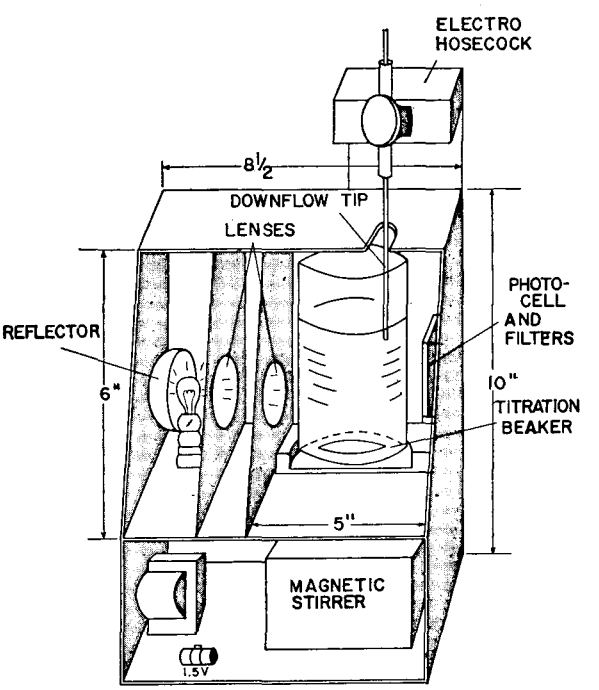


Figure 2. Titrator

Front removed

Ammonium chloride–methyl red solution. Add 10 ml. of 0.02% methyl red solution to 1 liter of 15% ammonium chloride.

Sodium tungstate, 20% in water.

Murexide indicator solution, approximately 0.2% in water. This solution should not be kept for more than 3 days.

Eriochrome Black T indicator solution, approximately 0.2% solution in water. This solution should not be kept more than 3 days.

Aluminum chloride solution. Dissolve 133 mg. of aluminum foil or ribbon in hydrochloric acid and dilute to 500 ml. A 1-ml. aliquot contains the equivalent of 0.5 mg. of aluminum oxide.

Standard calcium oxide solution. Transfer 1.000 gram of Bureau of Standards standard sample No. 88 (dolomite) to a 250-ml. beaker. Add 20 ml. of hydrochloric acid (1 + 1), cover, and boil for 3 to 5 minutes. Cool the solution to room temperature and dilute to 1 liter in a volumetric flask. Ten milliliters of solution contain the equivalent of 3.05 mg. of calcium oxide.

Titration beakers, 400-ml., tall-form beakers with a line marked off about 3.5 inches from the bottom of each beaker.

Plastic-covered stirring bars. Several should be available.

STANDARDIZATIONS

It is necessary to standardize both the dilute Versene and the concentrated Versene. The dilute Versene is delivered in the titrator and is finally standardized in terms of milligrams of calcium oxide per second of flow or as milligrams of magnesium oxide per second flow. The concentrated Versene is delivered by an ordinary buret and is standardized in terms of milligrams of calcium oxide per milliliter of Versene or milligrams of magnesium oxide per milliliter of Versene.

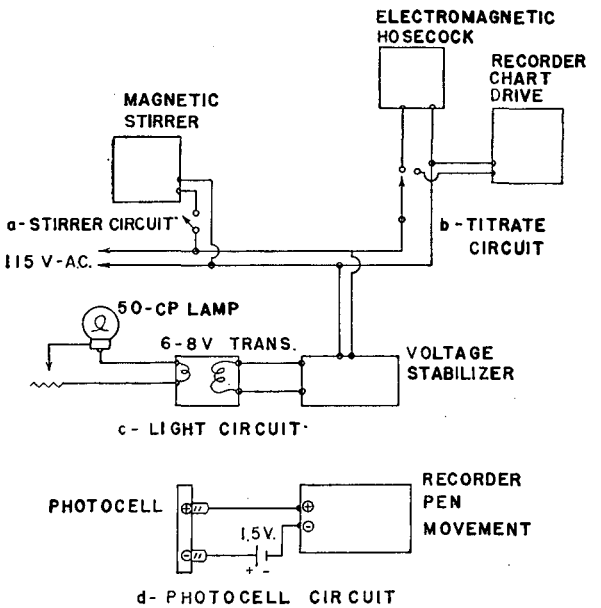


Figure 3. Circuitry of titrator

Standardization of Dilute Versene Solution. 1. Transfer 1 ml. of the calcium oxide standard solution to a 400-ml., tall-form titration beaker.

2. Add water to the mark on the beaker, then add 10 ml. of 15% sodium hydroxide.

3. Place a stirring magnet in the beaker and add 1 ml. of the murexide indicator solution.

4. Place the beaker in position within the titrator with both the green filter and the orange filter in position in front of the photocell.

5. Insert the downflow tip into the liquid, and turn the stirrer switch to "on."

6. Turn the light-control rheostat until the recorder pen indicates nearly maximum.

7. Throw the titrate switch to "titrate." This de-energizes the electromagnetic hosecock, allowing Versene to flow, while at the same time the recorder is energized and the chart proceeds to move at a fixed rate.

8. As the titration proceeds the pen that is tracing out the curve moves to the left as the indicator changes color (Figure 1). When sufficient Versene has flowed into the beaker to react completely with the calcium, the pen no longer moves to the left but traces a line parallel with the chart movement. After about 1 inch of this straight portion is traced out, reset all switches to "off."

9. Repeat the procedure from 1 through 8, using a 10-ml. portion of the standard solution.

10. Use the curves to obtain the end point of the titrations as illustrated in Figure 4. Extend a ruled line from the last portion of the curve, the flat portion, and extend another ruled line along the portion of the curve immediately preceding the sudden change of direction. The intersection of these lines is the end point. The time required in seconds for the titration is measured from the starting point to the end point.

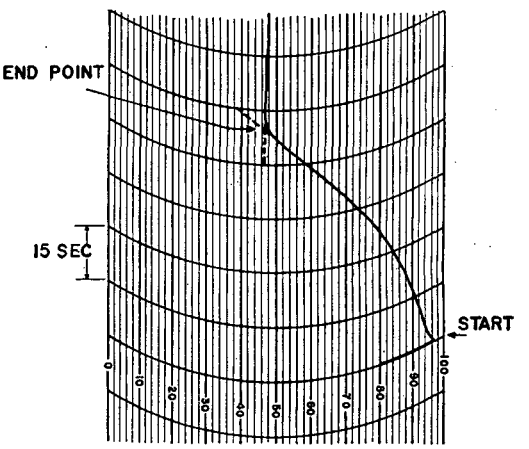


Figure 4. Titration curve

11. Subtract a blank correction from each titration. It is obtained by dividing the total number of seconds for the 10-ml. aliquot by 10 and subtracting the result from the number of seconds for the 1-ml. aliquot. This procedure is better than the direct titration of a blank solution, as the curve for a blank solution is more poorly defined than one for a solution containing some calcium. The blank is a small number, and when obtained in this manner it differs from a theoretical blank by a negligible amount. It is essentially the same value for calcium oxide and magnesium oxide.

12. Complete the standardization of the dilute Versene with the following calculation:

$$\frac{3.05}{(\text{Seconds for 10-ml. standard}) - \text{blank}} = \text{mg. of CaO per second}$$

13. Then,

$$\text{Mg. of CaO per second} \times 0.719 = \text{mg. of MgO per second}$$

The use of this factor has been confirmed with pure magnesium.

Standardization of Concentrated Versene Solution. 1. Transfer 25 ml. of the standard solution to a 400-ml. titration beaker.

2. Add about 200 ml. of water, 10 ml. of 15% sodium hydroxide, and 1 ml. of the murexide indicator solution.

3. Pour 10 to 20 ml. of the solution into a small beaker and set aside.

4. By means of a buret add the concentrated Versene solution rapidly until the end point is definitely passed (the indicator changes from salmon to purple).

5. Read the buret carefully and record the reading.

6. Replace the solution in the small beaker in the main portion of the solution; the color should change back to salmon.

7. Add water to the mark on the beaker, place it in the titrator, and complete the titration as in the standardization of dilute Versene solution above, steps 3 through 8.

8. Translate the curve obtained into seconds.

9. Subtract the "blank" correction.

10. Multiply the result by the value obtained for the standardization of dilute Versene. The answer is then milligrams of calcium oxide titrated by dilute versene.

11. To complete the standardization of the concentrated Versene perform the calculation:

$$\frac{(7.63 \text{ mg.}) - (\text{mg. titrated by dilute Versene}) (\text{step 10})}{\text{Buret reading}} = \text{mg. of CaO per ml. concd. Versene}$$

12. Then,

Mg. of CaO per ml. × 0.719 = mg. of MgO per ml. concd. Versene

PREPARATION OF SAMPLE SOLUTION

The choice of method of decomposing carbonate rock samples varies somewhat with the problem and the material to be analyzed. Generally, interest is centered upon the acid-soluble calcium and magnesium. The procedure for this decomposition is as follows:

1. Transfer 0.500 gram of sample to a 250-ml. beaker.
2. Add 20 ml. of hydrochloric acid (1 + 3) and boil 3 to 5 minutes.
3. Cool to room temperature and make to volume in a 250-ml. volumetric flask.

If desired, the calcium and magnesium in the insoluble portion may be recovered by filtering off the insoluble residue, burning the paper off in a platinum crucible, decomposing the residue with a few milliliters of hydrofluoric and sulfuric acids, fuming to dryness, and then taking the residue into solution with hydrochloric acid. The resulting solution is added to the filtrate from step 3, above, prior to dilution to 250 ml.

DETERMINATION OF CALCIUM

The determination of calcium proceeds in a manner analogous to the standardization of concentrated Versene solution.

Check to be sure that both color filters are in place.

1. Transfer 10 ml. of sample solution (equivalent to 20 mg. of sample) to a 400-ml. titration beaker.
2. Follow steps 2 through 10 in the section "Standardization of concentrated Versene solution." This yields milligrams of calcium oxide in that part of the aliquot titrated by the automatic titrator.
3. Multiply the buret titration by the standardization value for the concentrated Versene to obtain milligrams of calcium oxide in that part of the aliquot titrated by the buret.
4. Add the value for milligrams of calcium oxide obtained by the automatic titrator (step 2) to the value for milligrams of calcium oxide obtained by buret titration to get total milligrams of calcium oxide in the aliquot.
5. Calculate per cent calcium oxide by use of the formula:

$$\frac{\text{Total mg. of CaO in the aliquot} \times 100}{20} = \text{per cent CaO}$$

DETERMINATION OF MAGNESIUM

Check to be sure that only the orange filter is in place.

1. Transfer 25 ml. of sample solution (equivalent to 50 mg. of original sample) to a 250-ml. volumetric flask.
2. Add about 200 ml. of water, 1 ml. of aluminum chloride solution, and 15 ml. of the ammonium chloride–methyl red indicator solution, then ammonium hydroxide (1 + 1) dropwise until the indicator turns yellow.
3. Make the solution to volume, mix, and let stand 15 minutes.
4. Pour the solution through a dry filter paper in a dry funnel, catching 200 ml. of the filtrate in a dry 200-ml. volumetric flask.
5. Transfer the contents of the flask to a 400-ml. beaker and rinse the flask with distilled water.
6. Add 10 ml. of the buffer solution and 10 ml. of the 20% sodium tungstate solution to the solution in the beaker.
7. Cover the beaker and transfer to a hot plate.
8. Bring the solution to a boil, allow to boil for approximately 2 minutes, and then cool to room temperature in a water bath.
9. Decant the solution into a 400-ml. titration beaker without rinsing. The major portion of the precipitated calcium tungstate will adhere to the sides of the beaker. The amount of solution left on the walls is inconsequential, and the extensive transfer of the tungstate precipitate is undesirable because the resulting cloudy solution diminishes the quality of the titration. Small quantities of precipitate are of no consequence.
10. Add 1 ml. of Eriochrome Black T indicator solution, and proceed with the titration as in standardization of concentrated Versene solution, steps 3 through 10, which provides the value for milligrams of magnesium oxide in that part of the aliquot titrated with the automatic titrator.
11. Multiply the buret value by the value for the standardization of concentrated Versene. This provides milligrams of magnesium oxide in the part of the aliquot titrated by the buret.
12. Add the value for magnesium oxide (mg.) obtained by the automatic titrator to the value for magnesium oxide (mg.) obtained by buret titration to get magnesium oxide in the 200/250 part of the aliquot (this is equivalent to 40 mg. of original sample).
13. Calculate per cent of magnesium oxide by use of the formula:

$$\frac{\text{Total mg. of MgO} \times 100}{40} = \text{per cent MgO}$$

DISCUSSION OF RESULTS

To provide information as to the accuracy and precision of the procedure, a set of mixtures was carefully prepared using National Bureau of Standards standard sample 88 (dolomite) and pure calcium carbonate to cover the range of concentrations of calcium and magnesium that may occur in limestones and dolomites. The range of calcium varied from 1.5 to over 100 times the magnesium content. The set was run through twice, on different days by different operators using different solutions of Versene and different breakups for each set. National Bureau of Standards standard samples 1A (limestone) and 88 (dolomite) were run with the set in the same manner except that No. 1A was treated with hydrofluoric and sulfuric acids to get the portion insoluble in dilute hydrochloric acid into solution.

The results are shown in Table I. The value of statistical treatment of such limited data is dubious. The data indicate that there is no significant bias in either procedure as deviations occur in both positive and negative directions.

Table I. Analyses of Known Mixtures

	Calcium Oxide, %			Magnesium Oxide, %		
	"True"	Run 1	Run 2	"True"	Run 1	Run 2
A	55.5	55.8	55.8	0.40	0.42	0.44
B	53.6	53.9	53.9	2.0	2.1	2.0
C	51.1	51.2	51.4	4.1	4.2	4.1
D	48.4	48.3	48.7	6.5	6.6	6.4
E	45.8	45.8	46.0	8.6	8.4	8.6
F	43.2	43.3	43.4	10.8	10.7	10.9
G	40.7	40.7	40.7	12.9	13.0	13.1
H	38.1	38.0	38.1	15.1	15.1	15.2
I	35.6	35.4	35.6	17.2	17.0	17.3
J	55.8	55.6	55.9	0.21	0.23	0.23
NBS 1A	41.3	40.9	40.9	2.2	2.3	2.0
NBS 88	30.5	30.4	30.4	21.5	21.7	21.6

The Versene method described in this paper has the advantage of greater speed over conventional gravimetric procedures. In addition, the automatic titrator removes the subjective evaluation of end points, which often limits the desirability of Versene procedures. The titrator described is inexpensive and simple to construct, the major cost being the milliammeter recorder. If available, the titration can be made in any type of spectrophotometer for which a recorder can be adapted.

LITERATURE CITED

(1) Banewicz, J. J., and Kenner, C. T., ANAL. CHEM., 24, 1186 (1952).
(2) Barredo, J. M. G., and Taylor, J. K., *Trans. Electrochem. Soc.*, 192, 437 (1947).
(3) Cheng, K. L., Kurtz, T., and Bray, R. H., ANAL. CHEM., 24, 1640 (1952).
(4) Sweetser, P. B., and Bricker, C. E., *Ibid.*, 25, 253 (1953).

RECEIVED for review November 17, 1954. Accepted January 18, 1955. Publication of this paper is authorized by the director, U. S. Geological Survey.

Polarographic Determination of Copper, Nickel, Cobalt, Manganese, and Chromium in Titanium Alloys

JAMES J. MIKULA and MAURICE CODELL

Pitman-Dunn Laboratories, Frankford Arsenal, Philadelphia, Pa.

Work was initiated in this laboratory to test the applicability of the polarograph to the determination of constituents of titanium alloys. This paper describes accurate and fairly rapid polarographic methods for determining copper, nickel, cobalt, manganese, and chromium. Copper, nickel, and cobalt are determined simultaneously in a pyridine–pyridinium chloride supporting electrolyte after hydrolyzing most of the titanium from a perchloric acid solution. The pH of the solution before polarographing is 5 to 5.5. Manganese is determined after a dilute hydrofluoric acid solution has been adjusted to a pH of approximately 6.6 by the addition of barium carbonate, titanium being precipitated during the neutralization. Chromium is determined from a sodium hydroxide solution after oxidation of the chromium to the hexavalent state with ammonium persulfate and alkaline peroxide. These methods have been found satisfactory for determining 0.2 to 5.0% copper, nickel, or cobalt in the presence of each other, 1 to 10% manganese, and 0.05 to 20% chromium, using a sample weight of 0.1 gram.

VERY little work has been reported on the polarographic analysis of titanium and its alloys. As the importance of titanium and its alloys has greatly increased during the past few years, it was considered advisable to study the possibility of polarographic methods for analyzing these alloys.

Recently this laboratory has developed polarographic methods for the determination of molybdenum (*1*) and aluminum (*11*) in titanium alloys. This paper deals with the determination of copper, nickel, cobalt, manganese, and chromium.

Many investigators have developed polarographic procedures for the determination of copper, nickel, and cobalt in steel and ferroalloys. Thanheiser and Maasen (*9, 16*) determined copper and nickel simultaneously from an ammoniacal ammonium chloride solution after removing iron. Stackelberg, Klinger, Koch, and Krath (*14*) also recommended an ammoniacal electrolyte for the determination of cobalt and nickel.

Thanheiser and Maasen (*15*) employed a barium chloride electrolyte for the simultaneous determination of nickel and cobalt. An attempt to use either ammoniacal ammonium chloride or a barium chloride supporting electrolyte for analyzing titanium alloys was found to be unfeasible because the half-wave potentials of nickel and cobalt are so close that their waves tend to coalesce, especially when the concentrations of nickel and cobalt are disproportionate. An accurate measurement of their wave heights is therefore impractical. Lingane and Kerlinger (*6*) studied the simultaneous determination of nickel and cobalt in supporting electrolytes of pyridine and thiocyanate. In these electrolytes the half-wave potential of nickel is 0.3 volt more positive than that of cobalt, and this excellent separation of the two waves permits the simultaneous determination of both metals. Lingane and Kerlinger (*6*) recommend the use of the pyridine supporting electrolyte in preference to the one containing thiocyanate because the diffusion current of cobalt shows peculiar irregularities with thiocyanate. The authors found that pyridine was an excellent supporting electrolyte for the simultaneous determination of copper, nickel, and cobalt in titanium base alloys. The bulk of the titanium is removed by hydrolysis in acid solution; the remainder is precipitated by the addition of excess pyridine prior to polarographing the solution. No appreciable quantity of copper, nickel, or cobalt is coprecipitated with the titanium during the hydrolysis or addition of pyridine.

Prajzler (*12*) and Stackelberg, Klinger, Koch, and Krath (*14*) have shown that a barium chloride supporting electrolyte is suitable for the determination of manganese. The latter group employed the barium chloride supporting electrolyte for the determination of manganese in manganese steels. Barium salts proved to be satisfactory for the analysis of titanium alloys because the titanium can be precipitated by the neutralization of a dilute hydrofluoric acid solution using barium carbonate. Barium fluoride appears to be a suitable electrolyte for determining manganese.

The reduction of chromate produces a well-defined wave in 0.1 to 1*N* sodium hydroxide. According to Lingane and Kolthoff (*7*), 1*N* sodium hydroxide is the most suitable electrolyte for the polarographic determination of chromate. Several investigators (*14, 17*) employed this electrolyte to determine chromium in steel and ferroalloys. The authors found that sodium hydroxide serves as a satisfactory electrolyte for determining chromium in titanium. Titanium is partially removed by hydrolysis from a boiling dilute sulfuric acid solution, after which chromium is oxidized to chromate. Iron must be added to the sample to ensure that the last traces of titanium are precipitated by the sodium hydroxide.

Table I. Effect of Chromium on Determination of Copper in Pyridine–Pyridinium Chloride Supporting Electrolyte

Copper Added, %	Chromium Added, %	Copper Found, % Av.
5.00	0.0	4.98
5.00	2.0	5.42
5.00	5.0	5.68
5.00	10.0	5.84
5.00	10.0[a]	4.97
1.00	10.0[a]	1.02

[a] Removed by precipitating with $BaCl_2$.

EXPERIMENTAL

In developing the method for copper, nickel, and cobalt, the authors at first attempted to dissolve the sample with hydrofluoric acid in platinum ware, followed by evaporation to fumes of perchloric acid to hydrolyze the bulk of the titanium. This procedure was not satisfactory, however, because the cobalt wave was obscured by the discharge of hydrogen. A series of controlled tests showed that a trace quantity of platinum is dissolved during the fuming of perchloric acid, thus causing a marked increase of the hydrogen overpotential. This catalytic hydrogen wave with platinum has been studied by Šlendyk (*13*) and Herasymenko (*2, 3*). Because of this interference it was necessary to dissolve the sample in glassware. Attempts to dissolve the sample in a mixture of fluoboric and perchloric acids were unsuccessful. The use of dilute hydrofluoric and perchloric acids was found to be a convenient and satisfactory means of dissolving the sample. Only slight etching of the beaker resulted from this procedure and further use of the beakers was not impaired.

Chromium interfered with the determination of copper (Table I). In steel, trivalent chromium can be coprecipitated with iron, using pyridine (*19*). Since the chromium was present as the chromate after fuming with perchloric acid, a solution of barium chloride was added prior to the addition of the pyridine and gelatin. The barium chromate formed was quickly carried down by the hydrous titanium oxide upon the addition of pyridine.

Cobalt exhibits a pronounced maximum in a mixture containing 0.5*M* pyridine and 0.5*M* pyridinium chloride (*6*). It was found that a final concentration of 0.05% gelatin was required before this maximum was effectively eliminated. Concentrations of gelatin in excess of 0.01% should be avoided, if possible (*5*); however, 0.05% gelatin did not lower the wave height appreciably and satisfactory results can be obtained if the quantity of gelatin is kept constant (*6*). In developing the manganese procedure the authors decided to employ hydrofluoric acid to dissolve the sample, since titanium is so readily soluble in this acid. The titanium was precipitated as the hydrous oxide by the addition of barium carbonate. In order to prevent partial coprecipitation of the manganese, it was found necessary to keep the solution cooled below 20° C. during the neutralization. It was unnecessary to filter the solution prior to polarographing. The precipitate settled rather quickly and a portion of the supernatant liquid was polarographed. The addition of a small quantity of gelatin was necessary to suppress the slight maximum exhibited by the manganese wave.

In developing the chromium procedure, the authors found that some titanium remained in solution after the addition of the excess sodium hydroxide. The titanium exhibited a reduction wave which began just before the limiting current of the chromate was reached, thus making a measurement of the chromium wave impossible. When titanium alone is precipitated with sodium hydroxide the precipitation is not complete, but when iron accompanies it, all the titanium is precipitated (*4*). It was therefore decided to incorporate a small amount of open-hearth iron to the original sample. This procedure worked satisfactorily and the interference of the titanium wave was eliminated. Consideration was given to the possibility of precipitating most of the titanium prior to adding sodium hydroxide by boiling it in a dilute solution of sulfuric acid. It was found that no chromium is occluded; therefore, this step was included in the procedure. Originally the oxidation of chromium by ammonium persulfate was performed from solution of dilute hydrofluoric acid, but titanium forms a complex with hydrofluoric acid and is not precipitated upon boiling. It was therefore decided to dissolve the sample using hydrofluoric, nitric, and sulfuric acids in platinum and to evaporate to fumes of sulfuric acid. Only a few minutes were required for the entire procedure. The complete oxidation of chromium was supplemented by the use of alkaline peroxide, since 100% conversion was not achieved with ammonium persulfate and silver nitrate alone. The presence of manganese causes interference in the chromate determination by exhibiting a reduction wave that coalesces with the chromate wave, causing an increase in the wave height and consequently high results. Interference from manganese was effectively removed by reducing the permanganate with hydrochloric acid, precipitating with ammonium hydroxide, and filtering the precipitated iron, titanium, and manganese. Any remaining manganese is removed by boiling in ammoniacal bromine solution, and filtering.

Calibration Curves and Constants. The calibration curves for copper, nickel, and cobalt (Figure 1) were prepared by adding known amounts of standard copper, nickel, and cobalt solutions, respectively, to pure titanium, and carrying them through all the steps of the procedure. The capillary used had an m value of 1.94 mg. sec.$^{-1}$ The drop times were measured in the supporting electrolyte used in the procedure at applied potentials equal to the potentials at which the reduction waves were measured. The t values were found to be 3.03, 3.12, and 2.73 seconds, respectively, for copper, nickel, and cobalt. The $m^{2/3}t^{1/6}$ values were 1.88 mg.$^{2/3}$ sec.$^{-1/2}$ for copper, 1.89 mg.$^{2/3}$ sec.$^{-1/2}$ for nickel, and 1.84 mg.$^{2/3}$ sec.$^{-1/2}$ for cobalt. The half-wave potentials ($E_{1/2}$) for the reduction of copper, nickel, and cobalt were found to be −0.25, −0.78, and −1.06 volts *vs.* the S.C.E.

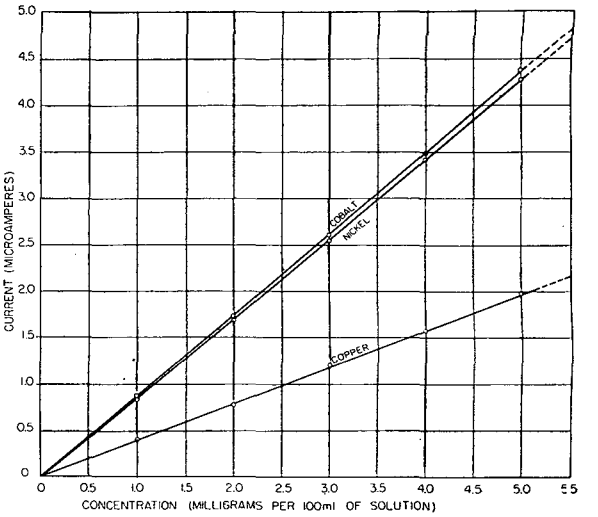


Figure 1. Relation between diffusion current and concentration of copper, nickel, and cobalt

A series of standards for the determination of manganese was prepared by adding known amounts of a standard manganese solution to a 100-ml. volumetric flask containing the proper amount of supporting electrolyte and gelatin, diluting to volume, and polarographing. The diffusion current was measured just beyond the full development of the reduction wave for manganese at −1.7 volts *vs.* S.C.E. The relationships id/C, and $id/Cm^{2/3}t^{1/6}$ were calculated and found to be constant (Table II). All the results reported for manganese were based on the use of these constants. The half-wave potential for manganese was found to be −1.54 volts *vs.* the S.C.E.

The relationships id/C and $id/Cm^{2/3}t^{1/6}$ were also established for the reduction of chromium (Table III) by adding known amounts of standard chromate solution to a 50-ml. volumetric flask containing 1 ml. of 0.5% gelatin and 1 ml. of sulfuric acid, neutralizing with 4*N* sodium hydroxide, adding 20 ml. in excess, and polarographing the resulting solution. Separate standards

Table II. Relation between Diffusion Current and Concentration of Manganese

(Diffusion currents measured at −1.7 volts *vs.* S.C.E.; h = 79 cm.; m 1.94 mg. sec.$^{-1}$; t = 2.44 sec.; $m^{2/3}t^{1/6}$ = 1.82 mg.$^{2/3}$ sec.$^{-1/2}$)

C, Millimoles/ Liter	id, μa.	id/C	$id/Cm^{2/3}t^{1/6}$
0.0364	[a]	...	...
0.182	1.06	5.82	3.19
0.364	2.13	5.85	3.21
0.546	3.20	5.86	3.21
0.728	4.27	5.87	3.22
0.910	5.40	5.93	3.25
1.820	10.80	5.93	3.25
		Av. 5.88	3.22

[a] Interference from barium wave made accurate measurement impossible

Table III. Relation between Diffusion Current and Concentration of Chromium

(Diffusion currents were measured at −1.1 volts *vs.* S.C.E.; h = 79 cm.; m = 1.94 mg. sec.$^{-1}$; t = 3.70 sec.; $m^{2/3}t^{1/6}$ = 1.93 mg.$^{2/3}$ sec.$^{-1/2}$)

C, Millimoles/ Liter	id, μa.[a]	id/C	$id/Cm^{2/3}t^{1/6}$
0.03846	0.348	9.05	4.68
0.3846	3.56	9.26	4.79
0.7692	7.10	9.23	4.77
1.154	10.7	9.27	4.79
1.539	14.1	9.16	4.74
1.923	17.6	9.15	4.73
3.846	35.4	9.20	4.76
7.692	70.8	9.20	4.76
		Av. 9.19	4.75

[a] Corrected for residual current of supporting electrolyte alone.

should be set up for the chromium determination in the presence of manganese. Standard chromate solution is added to a 50-ml. volumetric flask containing 1 ml. of sulfuric acid, and 1 ml. of hydrochloric acid, which has been neutralized with sodium hydroxide with the addition of 20 ml. of 4*N* sodium hydroxide in excess. One milliliter of 0.5% gelatin solution is added and the resulting solution is polarographed after being diluted to volume. The relationships id/C and $id/Cm^{2/3}t^{1/6}$ are calculated from the height of the wave.

The standard solutions used for the calibrations were prepared as follows:

Standard copper solution (1.00 ml. = 1.00 mg. of copper). Prepared by dissolving 1.0000 gram of pure copper powder in a minimum amount of nitric acid and diluting to 1 liter with water.

Standard nickel solution (1.00 ml. = 1.00 mg. of nickel). Prepared by dissolving 4.0501 grams of C.P. nickel chloride hexahydrate in 1 liter of water.

Standard cobalt solution (1.00 ml. = 1.00 mg. of cobalt). Prepared by dissolving 4.0372 grams of C.P. cobalt chloride hexahydrate in 1 liter of water.

Standard manganese solution (1.00 ml. = 1.00 mg. of manganese). Prepared by dissolving 1.0000 gram of electrolytic manganese in 5 ml. of perchloric acid (70%) with heat, and diluting to 1 liter with water.

Standard chromium solution (1.00 ml. = 1.00 mg. of chromium). Prepared by dissolving 2.8284 grams of National Bureau of Standards potassium dichromate in 1 liter of water.

APPARATUS AND REAGENTS

A Sargent polarograph, Model XXI, was used in performing this work. Electrolysis was carried out in an H-type polarographic cell as described by Lingane and Laitinen (*8*). The cell was kept in a water thermostat at 25° ± 0.1° C. Dissolved oxygen was removed by bubbling purified nitrogen through the solution for 10 minutes prior to the polarographic determinations. Vanadous sulfate (*10*) was used to purify the nitrogen.

No Bump solution evaporators (Moroney) secured from Fischer Scientific Co., Catalog No. 2-542.

Platinum dishes or crucibles, 30-ml. capacity or larger.

Plastic dropper, polyethylene or polystyrene.

Pyridine, reagent grade, boiling range 113° to 116° C.

Hydrofluoric acid, C.P. 48%.

Perchloric acid, 72%.

Hydrochloric acid, specific gravity, 1.19.

Sulfuric acid (20%). Prepared by carefully adding 20 ml. of sulfuric acid to 80 ml. of water.

Gelatin solution (1%). Prepared by dissolving 1.00 gram of gelatin with approximately 50 ml. of water by applying low heat, transferring to a 100-ml. volumetric flask, and diluting to mark. This solution should be freshly prepared before using.

Gelatin solution (0.5%). Dilute a portion of 1% gelatin solution with an equal volume of water.

Barium chloride solution (10%). Dissolve 10 grams of barium chloride in 100 ml. of water.

Barium hydroxide (saturated solution). Saturate about 100 ml. of water with barium hydroxide by shaking.

Barium carbonate, C.P. powder.

Iron, open-hearth or other chromium-free iron.

Silver nitrate solution (0.25%). Dissolve 0.25 gram of silver nitrate in 100 ml. of water.

Ammonium persulfate, C.P.

Sodium hydroxide (4*N*). Dissolve 80 grams of sodium hydroxide in 500 ml. of water.

Hydrogen peroxide, 30%.

Graphite, chromium-free.

PROCEDURES

Copper, Nickel, and Cobalt. Place a 0.1-gram sample in a 250-ml. beaker containing approximately 15 ml. of water. Using a plastic dropper, add 15 to 20 drops of hydrofluoric acid, place on hot plate, and warm gently until titanium is completely dissolved. Add 1 to 2 drops of nitric acid to oxidize the titanium. (A few additional drops of nitric acid may be required to dissolve possible alloying elements.) Add 2 ml. of perchloric acid, and evaporate to moist dryness, but do not bake. In order to ensure uniform heating and to prevent spattering, this operation should be performed in a No Bump solution evaporator (see apparatus). Remove from the hot plate and cool to room temperature. Add exactly 2.0 ml. of concentrated hydrochloric acid, and swirl to dissolve salts. Wash the sample into a 100-ml. volumetric flask using 50 to 75 ml. of water. (If chromium is present, add 5 ml. of 10% barium chloride solution at this point. If chromium is absent, the addition of barium chloride may be eliminated.) Add exactly 5.0 ml. of pyridine (approximately 13*M*), shake gently, and let stand 2 minutes; then add 5.0 ml. of 1.0% gelatin solution, dilute to mark with water, and mix. Allow the precipitate to settle, then polarograph an aliquot of the supernatant solution between 0.0 and −1.3 volts *vs.* the S.C.E. Measure the height of the waves whose $E_{1/2}$ occur at −0.25, −0.78, and −1.06 volts *vs.* the S.C.E. Correct for the residual current. From the diffusion currents, determine the copper and/or nickel and/or cobalt concentration from a calibration curve or from the relation $C = id/K$.

Manganese. To a 0.1 gram-sample contained in a platinum dish or crucible add approximately 5 ml. of water and a minimum amount of hydrofluoric acid (about 10 drops); heat gently until completely dissolved. Add 1 to 2 drops of concentrated nitric acid, and heat until the titanium is completely oxidized. Transfer the sample to a 100-ml. volumetric flask, and immerse in an ice bath for at least 5 minutes before proceeding. Add saturated barium hydroxide solution dropwise, until the appearance of the first persistent haziness. Add a few drops of methyl red indicator solution, then add barium carbonate powder until the supernatant solution just becomes a definite yellow. Add 2.0 ml. of 0.50% gelatin solution, dilute to the mark, mix, and polarograph a portion of the supernatant liquid over the range −1.3 to −1.8 volts *vs.* S.C.E. From the diffusion current, corrected for residual current, determine the manganese concentration by consulting a calibration curve or from the relation $C = id/K$.

Chromium. To a 0.1-gram sample contained in a platinum dish add 25 mg. of chromium-free iron, 5 ml. of 20% sulfuric acid, and 15 to 20 drops of hydrofluoric acid. Heat if necessary until the sample is dissolved. Add 2 to 3 drops of nitric acid, and heat the sample gently until fumes of sulfuric acid are evolved; continue heating 2 to 3 minutes longer, remove from hot plate, and cool. Using a stream of water, wash the sample into a 250-ml. beaker, and dilute to approximately 100 ml. Place on hot plate and bring the solution to a boil; boil for 5 minutes. (Titanium will hydrolyze at this point.) Add 5 ml. of silver nitrate solution, and cautiously add about 1 gram of ammonium persulfate. Boil 10 to 15 minutes to destroy the excess ammonium persulfate. (If the solution becomes pink, manganese is indicated and the modified procedure for chromium should be followed from this point. If no pink color results, continue as follows.) Remove from the hot plate and cool. Neutralize with 4*N* sodium hydroxide until all the iron is just precipitated. Add 20 ml. of 4*N* sodium hydroxide containing 25 to 30 drops of 30% hydrogen peroxide. Add a small amount of graphite to prevent bumping, and boil the solution until a volume of approximately 15 to 20 ml. remains. Cool and transfer the sample to a 50-ml. volumetric flask. Add 1.0 ml. of 0.50% gelatin solution, take to volume, and polarograph a portion of the supernatant liquid over the range −0.4 to −1.6 volts *vs.* S.C.E. From the diffusion current, after correcting for the residual current, determine the chromium concentration by consulting a calibration curve or calculating from the relation $C = id/K$.

Modified Procedure for Chromium in Presence of Manganese. Follow the chromium procedure up to and including the 10- to 15-minute boiling after the addition of ammonium persulfate. At this point add 1.0 ml. of concentrated hydrochloric acid, and boil for 10 minutes, filter on a No. 40 Whatman paper, and wash several times with hot water. Heat the filtrate to boiling, remove from the hot plate, and neutralize with 4*N* sodium hydroxide until a slight permanent precipitate appears. Add 20 ml. of ammonium hydroxide and 20 ml. of saturated bromine water. Boil for about 3 to 4 minutes, filter through No. 41 Whatman paper, and wash with warm 2% ammonium hydroxide solution. Boil the filtrate 10 to 15 minutes, then cautiously add 20 ml. of 4*N* sodium hydroxide containing 25 to 30 drops of 30% hydrogen peroxide. Boil 10 minutes, filter through No. 40 Whatman paper, and wash with water. Add a small amount of graphite to the filtrate to prevent bumping, and boil until a volume of approximately 15 to 20 ml. remains. Cool and transfer the sample to a 50-ml. volumetric flask. Add 1.0 ml. of 0.5% gelatin solution, dilute to volume, and polarograph a portion of the supernatant liquid over the range −0.4 to −1.6 volts *vs.* S.C.E. From the diffusion current after correcting for the residual current, determine the chromium concentration by consulting a calibration curve or calculating from the relation $C = id/K$ established by running standards in the supporting electrolyte used in the procedure.

RESULTS

As there were no reliable standards available for copper, nickel, cobalt, or manganese, it was necessary to check the accuracy and precision of the methods by using synthetic standards. The standards were prepared by adding known quantities of standard solutions containing copper, nickel, cobalt, and manganese salts to

Table IV. Analysis of Synthetic Titanium Samples Containing Copper, Nickel, and Cobalt

Elements Added, %	Found, % Average	Std. Dev., %	No. of Detns.
0.200 Copper	0.190	0.010	5
0.200 Nickel	0.204	0.011	
0.200 Cobalt	0.202	0.020	
2.00 Copper	1.99	0.024	7
1.00 Nickel	0.99	0.021	
1.00 Cobalt	1.01	0.022	
1.00 Copper	0.97	0.020	6
1.00 Nickel	1.01	0.009	
5.00 Cobalt	4.99	0.050	
5.00 Copper	5.05	0.059	7
1.00 Nickel	1.01	0.022	
2.00 Cobalt	2.01	0.024	
5.00 Copper	5.02	0.035	7
5.00 Nickel	5.02	0.038	
5.00 Cobalt	5.02	0.058	
3.00 Copper	3.01	0.027	6
5.00 Nickel	5.06	0.072	
1.00 Cobalt	1.00	0.014	

Table V. Analysis of Synthetic Titanium Samples Containing Manganese

Mn Added, %	Mn Found, % Av.	Std. Dev., %	No. of Detns.
1.00	1.01	0.025	5
2.00	1.99	0.021	5
4.00	4.03	0.042	5
5.00	5.04	0.047	6
10.00	10.03	0.107	5

Table VI. Analysis of Synthetic Titanium Samples Containing Chromium

Chromium Added, %	Chromium Found, % Av.	Std. Dev., %	No. of Detns.
0.050[a]	0.052	0.0013	5
0.10	0.102	0.0022	5
1.00	1.02	0.014	6
5.00	5.02	0.048	6
10.00	9.96	0.087	5
20.00	20.03	0.159	5

[a] Based on 0.2-gram sample.

Table VII. Analysis of Chromium-Titanium Alloys

Sample	Chromium Present, %[a]	Chromium Found, %
WA-3[b]	0.168	0.174
		0.170
		0.171
		0.166
		0.172
		Av. 0.170
WA-7[c]	2.15	2.20
		2.20
		2.19
		2.08
		Av. 2.17

[a] Average found by chromium task force, Metallurgical Advisory Committee on Titanium, Panel on Methods of Analysis, using colorimetric procedure submitted by Frankford Arsenal (*18*).
[b] Contains 0.3 C, 0.1 Cr, 0.3 Al, also Mn. Modified procedure used for this sample.
[c] Contains 0.07 C, 2.7 Fe, 2.1 Mo, 0.1 W, 0.06 N.

pure titanium metal. The results, including the standard deviations, are listed in Tables IV and V. The accuracy of the chromium method was tested by analyzing synthetic samples and chromium-titanium alloys. The results obtained are listed in Tables VI and VII. All results listed were obtained by using an original sample weight of 0.1 gram; however, 0.2-gram samples have been analyzed with comparable precision and accuracy.

DISCUSSION

None of the elements, with the exception of chromium, which would be expected to be found in commercial titanium alloys, interferes with the simultaneous determination of copper, nickel, and cobalt. The interference of chromium is easily removed by the addition of barium chloride (Table I). It can be seen from Table IV that satisfactory results are obtainable for copper, nickel, and cobalt over the range of 0.2 to 5.0%.

Table VIII. Effect of Various Ions on Manganese Determination

Element Added	Quantity Added, %	Manganese Added, %	Manganese Found, %
Aluminum	10	1.00	0.99
Boron	1	1.00	1.00
Chromium	10	1.00	1.06
Cobalt	10	1.00	0.55
	5	1.00	0.63
	4	1.00	0.66
	3	1.00	0.66
	2	1.00	0.90
	1	1.00	0.99
	10	5.00	5.09
	5	5.00	5.05
Copper	5	1.00	0.86
	4	1.00	0.95
Iron	10	1.00	0.95
Magnesium	5	1.00	0.99
Molybdenum	10	1.00	0.99
Nickel	10	1.00	0.47
	5	1.00	0.66
	4	1.00	0.75
	3	1.00	0.96
	10	5.00	5.03
	5	5.00	5.03
Phosphorus	1	1.00	0.99
Silicon	5	1.00	1.00
Tin	5	1.00	0.96
Tungsten	5	1.00	1.01
Vanadium	5	1.00	1.04

The manganese determination gives satisfactory results provided the concentration of manganese is greater than 0.182 millimole per liter (Table II). At lower concentrations, the initial discharge of the barium wave overlaps the reduction wave of manganese before its limiting current is reached. Table VIII lists the effect of a number of ions on the determination of manganese. It indicates that a concentration of cobalt greater than twice that of manganese leads to low results. Nickel, if present in concentrations greater than about three times that of manganese, interferes, and the concentration of copper must be less than four times that of manganese. None of the other elements listed caused any serious interference.

It has been reported that none of the metals found in steel interferes with the chromate determination using a sodium hydroxide supporting electrolyte (*14*). It is therefore not expected that any of the metals found in titanium alloys would interfere with the proposed procedure.

LITERATURE CITED

(1) Codell, M., Mikula, J. J., and Norwitz, G., ANAL. CHEM., **25**, 1441 (1953).
(2) Herasymenko, P., and Šlendyk, I., *Z. physik. Chem.*, **A149**, 123 (1930).
(3) *Ibid.*, **A162**, 223 (1932).
(4) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," p. 455, Wiley, New York, 1929.
(5) Kolthoff, I. M., and Lingane, J. J., "Polarography," Vol. I, p. 165, Interscience, New York, 1952.
(6) Lingane, J. J., and Kerlinger, H., IND. ENG. CHEM., ANAL. ED., **13**, 77 (1941).
(7) Lingane, J. J., and Kolthoff, I. M., *J. Am. Chem. Soc.*, **62**, 852 (1940).
(8) Lingane, J. J., and Laitinen, H. A., IND. ENG. CHEM., ANAL. ED., **11**, 504 (1939).
(9) Maasen, G., *Angew. Chem.*, **50**, 375 (1937).
(10) Meites, L., and Meites, T., ANAL. CHEM., **20**, 984 (1948).
(11) Mikula, J. J., and Codell, M., *Anal. Chim. Acta*, **9**, 467 (1953).
(12) Prajzler, J., *Collection Czechoslov. Chem. Communs.*, **11**, 588 (1939).
(13) Šlendyk, I., *Ibid.*, **4**, 335 (1932).
(14) Stackelberg, M. V., Klinger, P., Koch, W., and Krath, E., *Tech. Mitt. Krupp*, **2**, 59 (1939).
(15) Thanheiser, G., and Maasen, G., *Arch. Eisenhüttenw.*, **10**, 441 (1937).
(16) Thanheiser, G., and Maasen, G., *Mitt. Kaiser-Wilhelm Inst. Eisenforsch. Dusseldorf*, **19**, 27 (1937).
(17) Thanheiser, G., and Willems, J., *Ibid.*, **21**, 65 (1939).
(18) Vigo, S., Watertown Arsenal, Watertown, Mass., Task Force Reports on Determination of Various Elements in Titanium Alloys.
(19) Watters, J. I., and Kolthoff, I. M., IND. ENG. CHEM., ANAL. ED., **16**, 187 (1944).

RECEIVED for review February 27, 1954. Accepted January 6, 1955.

Elimination of Interferences in Flame Photometry

PAUL PORTER and GARRARD WYLD
Shell Development Co., Emeryville, Calif.

Some of the types of interferences encountered in flame photometry are reviewed. A comparison is made of the effectiveness of a radiation buffer, an internal standard, and a direct-injection burner as means of controlling certain types of interferences. Although these techniques are valuable, it is necessary to have considerable knowledge about the nature of the sample, and the most accurate analyses require standards which are very similar in composition to that of the sample.

PRIOR to the development of the technique of flame photometry, the determination of alkali metals was a difficult problem for analytical chemists. In general, it was necessary to remove all other metals from the sample before the alkali metals could be determined. Excellent methods have been worked out for this isolation of the alkalies, but none of them can be called rapid.

In the late twenties Lundegardh (*9*) demonstrated the usefulness of a flame excitation source for the quantitative spectrographic determination of the alkali metals and a number of other elements. Later workers (*6, 8, 11*), improving upon his burner design, were able to achieve a reproducibility and steadiness of emission from the flame that permitted the direct measurement of the intensities of the spectral lines with photoelectric cells or phototubes. The use of this technique in this country for the determination of the alkalies received an impetus with the construction by Barnes, Berry, Richardson, and Hood (*1*) of a simple inexpensive "flame photometer." In this instrument an aqueous solution of the sample was sprayed into the air supply of an ordinary Meker burner, so that a finely dispersed aerosol was carried into the flame. The light emitted by the flame passed through a filter to isolate a narrow portion of the spectrum in the vicinity of the wave length of interest, and fell upon a barrier layer photoelectric cell. The response of the cell was measured by a galvanometer. This very simple instrument aroused great interest among analytical chemists and a somewhat similar apparatus was manufactured commercially as the Perkin Elmer Model 18.

However, the instrument had many drawbacks, and was particularly sensitive to acid and salt interferences (*10*). Berry, Cappell, and Barnes soon developed an improved instrument using lithium as an internal standard (*2*). In this instrument two photocells were employed with a light-filter system which allowed only light in the vicinity of the lithium 671 mμ line to strike one cell, while light in the region of the sodium 589 mμ lines or the potassium 768 mμ line fell upon the other. By measuring the ratios of the sodium or potassium radiation to that of lithium, rather than the absolute values of the intensities, many of the effects of fluctuating aspiration rate, variations in flame temperature, and changes in emission due to acid and salt effects were avoided. Commercial instruments of this general type are the Janke, Barclay, Baird, and Process and Instruments flame photometers. The same internal-standard principle is used in the Perkin-Elmer Models 52A and 52C, which employ a prism monochromator in place of light filters.

Many modifications have appeared in the literature in recent years. Some workers have preferred to retain barrier layer photocells because of their simplicity, while others have increased the complexity by using the more sensitive phototubes and photomultiplier tubes. A variety of spray chambers have been used for introducing sample solutions into the burner gases, and the burners have been of various types using propane-air, illuminating gas–air, acetylene-air, acetylene-oxygen, and hydrogen-oxygen. The schematic diagram in Figure 1 is that of a typical spray chamber-burner assembly using acetylene-air. The air supply for the burner passes over an atomizer tube, forming a fine spray of sample solution in the large spray chamber. The larger droplets settle out and are drained off, or in some cases, are recycled to the atomizer; the small droplets remain suspended in the air stream and are carried into the burner tube where the air is mixed with the fuel gas and the combustible mixture fed into the flame.

The latest development in the field is a burner which forms a spray directly in the flame rather than in a spray chamber preceding the burner. This was introduced by Weichselbaum and Varney (*13*), whose burner design and flame photometer are marketed by the Fearless Camera Co. The same principle was used in a burner attachment designed for use with the Beckman Model DU and Model B spectrophotometers. A schematic diagram of the latter burner is shown in Figure 2. The oxygen fed to the flame passes over the tip of a fine capillary dipped into the sample solution, and causes an aspirator effect which produces a spray of liquid in the flame. Acetylene (or hydrogen) passing through the outer annulus provides the fuel for combustion. Neither the Weichselbaum-Varney flame photometer nor the Beckman instruments have the internal-standard feature.

Although the flame as an excitation source possesses important advantages such as simple spectra which do not require high resolving power for the isolation of the various lines, and steady emission not subject to the fluctuations observed in the arc or spark, it suffers from a number of serious interferences. It is convenient to think of three different types of interferences: overlapping of spectra or background interference, radiation interference, and anion or acid interference. Spectroscopists would break these down into several more classifications (*4, 5*). In practice, the only foolproof way to carry out flame photometry is to know the concentration of everything else in the sample and duplicate this composition in the standards which are used to calibrate the instrument. If one is to analyze a large number of

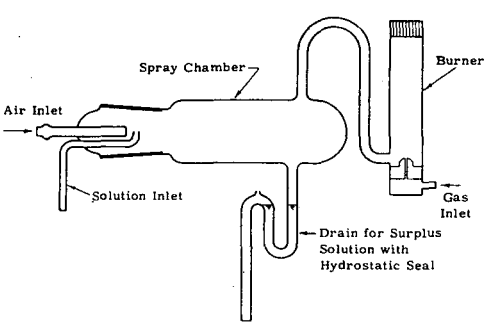


Figure 1. Spray chamber and burner assembly

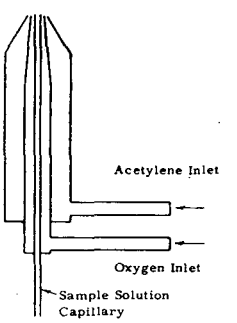


Figure 2. Burner to form sample spray directly in flame

samples of practically the same composition, except for variations in the sodium content, this is a very practical way to proceed. However, the analytical chemist is not always this fortunate so that it is desirable to find ways in which the various kinds of interferences can be minimized. Each type of interference is discussed below in terms of specific examples of special interest in the determination of sodium and potassium, the metals most frequently measured in flame photometry.

EXPERIMENTAL

A number of techniques are available for minimizing interferences in flame photometry without resorting to chemical separation or to duplication of the composition of the sample with standards. Several of these techniques are illustrated here, using three different types of instruments for comparison. A Perkin Elmer Model 52A instrument was used as an example of a direct-reading instrument with a spray chamber, and also as an example of an internal-standard instrument with a spray chamber, since it can be used either way. The Beckman Model B flame photometer was used as an example of a direct-reading instrument with the sample spray formed directly in the flame.

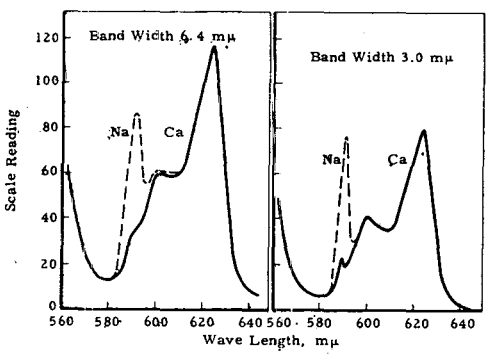


Figure 3. Effect of changing band width on calcium interference in determination of sodium

Beckman Model B flame photometer
— 0.02*M* calcium chloride containing trace of sodium impurity
--- 0.02*M* calcium chloride containing 5 p.p.m. of sodium

The Perkin-Elmer instrument had been slightly altered as follows: The feeding funnel and atomizer were replaced by a liquid intake capillary and atomizer assembly of the authors' own design, so constructed that the sample could be withdrawn from a beaker (Figure 1). A slight modification was also made in the burner to permit greater ease of cleaning. The instrument was equipped with a red-sensitive RCA phototube No. 918 or a blue sensitive RCA phototube No. 1P37 for measuring the unknown emission and a second red-sensitive tube for measuring the lithium internal standard emission.

In using the Perkin-Elmer photometer as a direct-reading instrument, a calibration curve was first prepared by measuring the emissions of pure sodium chloride solutions in distilled water or in the indicated buffer solutions of lithium chloride. With distilled water or a blank solution of lithium chloride buffer spraying into the burner chamber, the galvanometer zero control was adjusted to give a zero reading. A solution containing 20 p.p.m. of sodium was then drawn through the atomizer and the gain control was adjusted to bring the galvanometer reading to 100. Galvanometer readings were then made for solutions of sodium chloride at intervals of 2.5 p.p.m. of sodium through the range from 0 to 20. Before reading each solution, the settings for distilled water or buffer solution blank and the 20-p.p.m. solution were rechecked. Calibration curve intensities were redetermined several times during the course of the interference measurements, and were found to be highly repeatable. In applying the instrument to test solutions it was set with distilled water or blank solution and the 20-p.p.m. sodium standard as before, and an intensity reading was made on the test solution. The per cent error corresponding to the type of interference under test was then calculated by subtracting the known sodium content from the apparent value given by the calibration curve, multiplying by 100, and dividing by the known content.

A similar procedure was followed when the internal standard feature was used. The zero scale setting was made with pure lithium chloride solution, the 100-scale setting with 20-p.p.m. sodium in lithium chloride solution. Calibration points and interference data were obtained as for the direct procedure. In all instances, duplicate readings were made for the solutions.

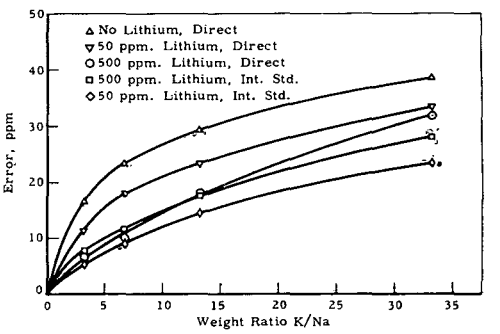


Figure 4. Effect of lithium chloride as a buffer on interference of potassium in determination of sodium

Perkin Elmer 52A, 15 p.p.m. of sodium

The Beckman flame photometer was operated with a red-sensitive phototube, Beckman No. 157, for wave lengths greater than 625 mμ, and with a blue-sensitive phototube, Beckman No. 12055, for wave lengths below 625 mμ. The burner was operated with acetylene gas and oxygen at pressures of 4 and 10 lb. respectively. The Beckman Model B spectrophotometer has four sensitivity positions, and is normally operated at highest sensitivity with the flame photometry attachment. Since no continuously variable gain control is provided in the amplifier, the setting of the 100 reference point must be made by varying the width of the slit. For each of the four sensitivity positions there is therefore a definite slit width which will give a scale reading of 100 with a given standard. In the present work calibration curves were prepared for the highest sensitivity position in the same manner as for the Perkin-Elmer instrument above. When it was desired to make readings for the same solutions at larger slit width, the photometer was operated one step lower in sensitivity.

The operating procedure was the following. With the shutter open the zero sodium or potassium standard was aspirated, and the dark current control was set to give a zero galvanometer reading. A 10-p.p.m. sodium standard or 20-p.p.m. potassium standard was then aspirated, and the slit width was adjusted to give an intensity reading of 100. Since there is some interaction between the zero and 100 settings, several successive readjustments are required to set the two ends of the scale when the blank solution has an appreciable emission. Test solutions were measured and calculated in the same way as for the direct Perkin-Elmer instrument. All measurements discussed in the next section are the result of at least two measurements.

With the Beckman Model B flame photometer it is possible to scan narrow regions of a spectrum to determine the nature of background radiation. For example, in the case of sodium determination, the wave-length dial is set for the 589 mμ lines in the usual way and the zero and 100 adjustments are made with blank and standard sodium solutions. The sample solution is then aspirated and readings of the galvanometer are taken as the wave-length dial is varied on both sides of the 589 mμ position. This procedure does not result in a curve giving the absolute intensity distribution in the flame since no compensation is made for variation of the phototube sensitivity with wave length; however, for the evaluation of interferences or the prediction of slit

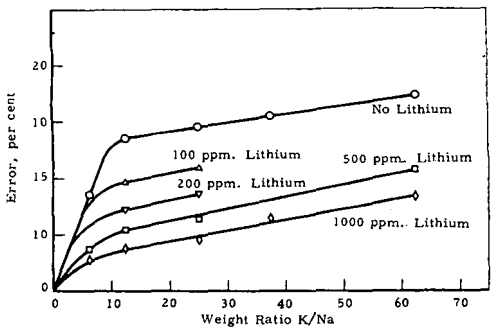


Figure 5. Effect of lithium chloride as a buffer in interference by potassium in determination of sodium

Beckman Model B, 8 p.p.m. of sodium Band width, 3 mμ

width effects it is the relative intensities obtained in the above manner which are of importance.

Standard solutions of the alkali halides and other solutions used were prepared from reagent grade chemicals. All salts and acids were examined for traces of the alkalies by applying the spectrum-scanning technique described above to concentrated solutions. Where sharp emission peaks were found at the alkali wave lengths, the corresponding amounts of the metals were estimated by the successive additions technique. Several small known increments of alkali were added to the solution and the changes in peak height were noted. The internal calibration thus afforded was used to calculate the amount which must have given rise to the original peak. In this manner it was possible to subtract contributions to the peak height of the alkali being determined from impurities in the solutions used in the interference tests.

DISCUSSION

The extent and character of the various kinds of interference are illustrated in Figures 3 to 9 and discussed below. Although the precision of the measurements was not established statistically, it was observed to be of the order of ±1 on the 0 to 100 scale of the instruments. This is equivalent, roughly speaking, to ±1 on the per cent error scale of Figures 4 to 9. These error curves indicate magnitude, direction, and character of interferences but should not be used as correction curves, because their magnitude may vary somewhat between instruments of the same make and even between burners in the same instrument.

Overlapping of Spectra. Calcium has a spectral band very near the best line for sodium (589 mμ). With a large amount of calcium present, sodium results will be high by an amount equal to the contribution of the calcium band to the total radiation passing through the slit when the monochromator is set at 589 mμ. This type of interference can be reduced by using a minimum slit width in spectrophotometers which have this adjustment (Figure 3) but cannot be eliminated entirely. The interference can be estimated, however, by means of a plot such as Figure 3 in which the background is drawn in across the base of the sodium peak and the appropriate value is subtracted from the total peak height. This type of interference measurement can be made fairly easily on the Beckman instruments. It is somewhat more difficult on the Perkin-Elmer instrument, because the wave-length scale is not finely enough graduated.

Radiation Interference. The presence of potassium or lithium, when sodium is being determined, will increase the radiation of the sodium, although the potassium and lithium themselves do not contribute radiation at this wave length. Sodium and lithium have a similar effect on the determination of potassium. This type of interference is believed due to the effect of one metal upon the ionization of the other metal in the flame. Thus sodium is believed to give off radiation in the flame at 589 mμ only when not ionized, and potassium is believed to repress the ionization of sodium and thus produce an enhancement of its radiation (*7*, *12*). The magnitude of the effect, however, is not proportional to the concentration of the interfering element. For example, in the determination of sodium, if some potassium is present, the addition of more potassium has much less effect than the same amount added to a pure sodium solution. Thus, if an analyst wished to determine sodium and did not know the concentration of potassium in the sample, he might add a large amount of potassium to both the sample and the standards and in this way minimize the effect of the potassium which was originally present in the sample. This has been called a buffering action because of its analogy to acid-base buffers (*14*). It has been shown that lithium will buffer the effect of sodium on potassium or of potassium on sodium (*3*). Thus it is not necessary that the buffering metal be the same as the interfering metal upon which it is acting.

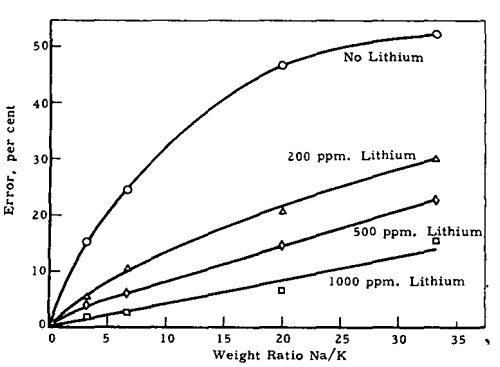


Figure 6. Effect of lithium chloride as a buffer on interference by sodium in determination of potassium

Beckman Model B, 15 p.p.m. of potassium Band width, 10.5 mμ

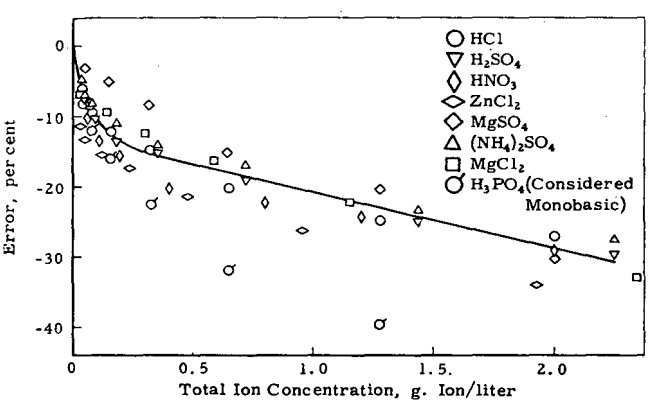


Figure 7. Errors in determination of sodium resulting from presence of acids and salts

Perkin-Elmer 52A, 15 p.p.m. of sodium

The interference of potassium in the determination of sodium is shown in Figure 4 for the Perkin-Elmer instrument. The use of a lithium chloride buffer is seen to diminish the interference markedly when the instrument is used to read the sodium emission directly. Here it is clearly seen that 500 p.p.m. of lithium is better than 50 p.p.m. However, when the internal-standard principle is used, 50 p.p.m. of lithium is more effective than 500 p.p.m.

The effectiveness of the internal-standard technique depends in

theory upon the interfering substance affecting the lithium and the sodium intensities to the same relative degree so that the intensity ratio is not affected. Apparently this requirement is more nearly reached when the lithium content is of the same order of magnitude as the sodium content.

Figure 5 shows the use of lithium chloride as a buffer for the interference of potassium in the determination of sodium with the Beckman Model B instrument. Figure 6 shows the effect of the same buffer on the interference of sodium in the determination of potassium. The Beckman instrument was not designed for use with an internal standard and it would be somewhat inconvenient to use it in that manner.

Anion Interference. If an appreciable amount of acid or salt is present in the determination of sodium with direct-reading, spray-chamber instruments, the radiation due to sodium is greatly reduced. It has been shown by Eggertsen, Wyld, and Lykken (*3*) that anion interference in this type of instrument is largely due to the effect of the acid or salt on the evaporation of solvent from the drops in the spray chamber. This, in turn, affects the amount of sample which reaches the flame without settling out.

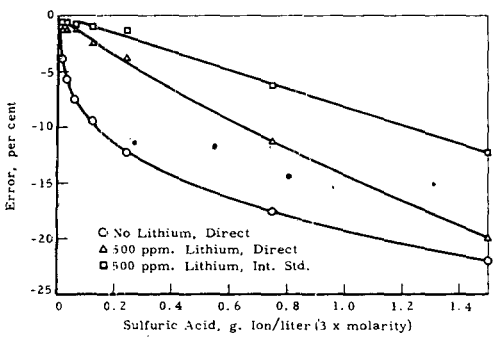


Figure 8. Reduction of sulfuric acid error by using lithium chloride buffer

Perkin Elmer 52A flame photometer, 8 p.p.m. of sodium

The interference of various acids and salts on the determination of sodium with the Perkin-Elmer direct-reading instrument is shown in Figure 7. The data extend those previously reported (*3*) and have been rechecked in the region previously covered. The solid line is drawn through the points for sulfuric acid. Plotting the concentration as gram-ions per liter gives the least scattering of points as would be expected if the interference were due to a vapor pressure effect. Gram-ions per liter is defined as the molarity multiplied by the number of ions per molecule in solution. Phosphoric acid appears to be an exception. Since the error increases most rapidly at low concentrations of the interfering compound, and since the principal interference of each compound is believed due to the same phenomenon of vapor pressure effect, any one of these compounds may be used to buffer the effect of any of the others. In practice, lithium chloride is used since it also serves as a radiation buffer and as an internal standard. Figure 8 shows the effect of lithium chloride in reducing the interference of sulfuric acid with the Perkin-Elmer instrument, using both the direct-reading and internal-standard techniques. The use of lithium chloride in the direct-reading instrument considerably diminishes the interference due to sulfuric acid; however, the use of lithium chloride as an internal standard decreases the interference even further.

Figure 9 shows the effect of various acids on the determination of sodium with the Beckman instrument. Since the spray is formed directly in the flame of this instrument, one would expect from the above discussion that there would be no vapor pressure effect and consequently less anion interference. Actually, the interferences are moderately large with certain acids and salts although the sharp increase in interference at low concentrations of interfering materials is not found with this instrument. The wide variation of interference among the various compounds with

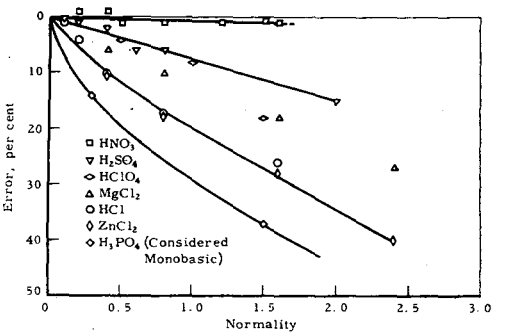


Figure 9. Errors in determination of sodium resulting from presence of salts and acids

Beckman Model B, 5 p.p.m. of sodium Band width, 3 mμ

the Beckman instrument suggests that, in some cases, an interaction with sodium is occurring within the flame which did not take place to the same extent in the cooler, more homogeneous flame of the Perkin-Elmer instrument. Also it is evident, from the absence of a sharp increase in interference at low concentrations of anion, that an anion buffer would not be effective with this instrument.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of Mrs. C. W. Manson who performed much of the experimental work reported in this paper.

LITERATURE CITED

(1) Barnes, R. B., Richardson, D. R., Berry, J. W., and Hood, R. L., ANAL. CHEM., **17**, 605–11 (1945).
(2) Berry, J. W., Cappell, D. G., and Barnes, R. B., *Ibid.*, **18**, 19–24, (1946).
(3) Eggertsen, F. T., Wyld, G. E. A., and Lykken, L., ASTM Special Technical Publication, **116**, pp. 52–65 (1951).
(4) Gilbert, P. T., Jr., *Ind. Lab.*, 3, 41–6 (1952).
(5) Gilbert, P. T., Jr., Hawes, R. C., and Beckman, A. O., ANAL. CHEM., **22**, 772–80 (1950).
(6) Heyes, Josef, *Angew. Chem.*, **50**, 871–4 (1937).
(7) Huldt, Lennart, "A Spectroscopic Study of the Electric Arc and the Acetylene-Air Flame," Dissertation, Uppsala, 1948.
(8) Jansen, W. H., Heyes, Josef, and Richter, C., *Z. physik. Chem.*, **A174**, 291–300 (1935).
(9) Lundegardh, H., "The Quantitative Spectral Analysis of the Elements," Jena, Gustav Fisher, Part I, 1929; Part II, 1934.
(10) Parks, T. D., Johnson, H. O., and Lykken, L., ANAL. CHEM., **20**, 822–5 (1948).
(11) Schuhknecht, W., *Angew. Chem.*, **50**, 299–301 (1937).
(12) Smit, J., Alkemade, C. T. J., and Verschure, J. C. M., *Biochim. et Biophys. Acta*, **6**, 508–21 (1951).
(13) Weichselbaum, T. E., and Varney, P. L., *Proc. Soc. Exptl. Biol. Med.*, **71**, 570–2 (1949).
(14) West, P. W., Folse, P., and Montgomery, D., ANAL. CHEM., **22**, 667–70 (1950).

RECEIVED for review November 2, 1954. Accepted January 15, 1955.

Infrared Evaluation of Sodium Salts of Organic Acids

EUGENE CHILDERS and G. W. STRUTHERS

Polychemicals Department, E. I. du Pont de Nemours & Co., Inc., Charleston, W. Va.

This investigation was undertaken to evaluate the feasibility of a method for analyzing mixtures of simple organic acids based on the infrared characteristics of their sodium salts. Determination of the acids themselves by infrared absorption is difficult and often impossible because of the effects of molecular association through hydrogen bonding. Infrared spectra of the sodium salts of the monobasic acids (acetic through caproic) and the dibasic acids (oxalic through pimelic) have been recorded over the 2- to 15-micron range. The spectral absorption bands of the sodium salts show a marked degree of specificity in contrast to the acids themselves, and can be used for both qualitative and quantitative evaluation of acid mixtures. A typical analysis—the determination of adipic acid in an adipic-glutaric acid mixture—is described in detail. A precision of 4% relative was obtained for the method at an adipic acid level of about 10%.

WHILE infrared absorption spectroscopy has been extensively applied to the analytical evaluation of many types of complex organic mixtures, little work has been reported on the determination of simple organic monobasic and dibasic acids. The analysis of multicomponent mixtures of acids by infrared absorptiometric methods is not normally attempted. Such mixtures exhibit a high over-all absorption throughout the commonly used infrared region (2 to 15 microns), which necessitates the use of very thin absorption cells. This often prohibits even qualitative evaluation of mixtures.

In addition to the generally opaque character of the acids to infrared radiation, the absorption bands obtained in the fingerprint regions are relatively nonspecific. Except where branching occurs in the carbon chain, the monobasic acid spectra are very similar. This phenomenon is even more pronounced in the spectra of the dibasic acids—succinic through adipic. Analytical evaluation of these complex acid mixtures is difficult because of lack of specificity.

These undesirable spectral characteristics are due primarily to intramolecular association through hydrogen bonding. Much theoretical investigation has been and is being conducted on the mechanism and spectral results from hydrogen bonding in acids (*1–3, 7, 10*).

One means of increasing the specificity of the analysis is by esterification of the acids prior to infrared measurement. The spectra of the resulting esters are generally far more specific for infrared characterization than the acids themselves. This procedure is necessarily time-consuming and is subject to errors from both incomplete alcoholysis and degradation during the distillation which usually follows alcoholysis. In spite of its limitations, this method has been widely applied, particularly in qualitative work.

Another approach to the analysis of acids is by evaluation of their metallic salts, whereby the effects of hydrogen bonding are eliminated. In this case the absorption spectrum obtained is that due to both the anion and the crystal structure of the salt. These absorption characteristics are influenced only slightly by the nature of the cation. In some isolated cases, the coprecipitated salts of a mixture of acids will not exhibit exactly the same spectra as those obtained when the pure salts are mechanically mixed—e.g., mixtures of disodium adipate and disodium succinate. This is doubtless related to the crystal structure of the coprecipitated material, since x-ray studies reveal the presence of new crystals. In these cases, a valid analysis can still be obtained after a reasonable knowledge of the constituents of the mixture has been procured.

In a series of papers (*4–6*), Duval, Lecomte, and Douvillé give an excellent theoretical discussion on the infrared spectra of metallic salts of mono- and dibasic acids. These papers deal primarily with the structure and modes of vibration of the carboxylate ion.

In the work described here, the sodium salts of the simple mono- and dibasic acids were examined from the standpoint of application to qualitative and quantitative analysis of acid mixtures. In almost all cases the salts were found to possess a number of sharp, specific bands entirely suitable for analytical purposes. Even acids with almost identical spectra gave quite dissimilar curves in the salt form.

PREPARATION OF SALTS FOR REFERENCE SPECTRA

In this work the anhydrous sodium salts of the monobasic acids, acetic through *n*-caproic, and of the dibasic acids, oxalic through pimelic, were prepared for the recording of reference spectra. The method of preparation was as follows.

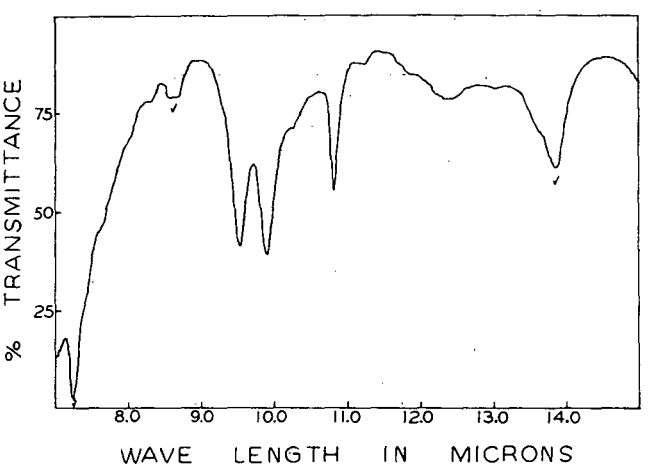


Figure 1. Sodium acetate

√. Mineral oil bands

Approximately 3 grams of the pure acid in 20 ml. of water (not necessarily dissolved) were neutralized with 25% aqueous sodium hydroxide and phenolphthalein indicator. The aqueous salt solution was evaporated slowly on a hot plate until a thick slurry of salt remained. The slurry was then cooled slightly and approximately 125 ml. of acetone were added with stirring to prevent lumping. The salt was filtered through a medium fritted-glass crucible, washed with 150 ml. of acetone, and dried in the crucible at 110° C. for 3 hours. Salts prepared in this manner contained no hydrates.

SAMPLING AND RECORDING OF REFERENCE SPECTRA

One of the undesirable features of this approach is the need for examination of the salts in the solid state, since most of the infrared bands seem to be related to the crystal nature of the materials. Furthermore, no satisfactory infrared solvent has

been found for the salts. Consequently, the conventional technique of mulling the salts in mineral oil was used.

Exactly 0.40 gram of the anhydrous salt was placed in an agate mortar and ground to a fine powder. Two milliliters of white mineral oil were added slowly from a 25-ml. buret while the mortar was being rotated to obtain maximum contact with the powder. The mixture was then mulled until a homogeneous paste was obtained. A portion of the paste was placed in a demountable-type sodium chloride cell manufactured by the Perkin-Elmer Co. A 0.025-mm. slotted spacer was used to permit escape of excess paste.

The 2- to 15-micron spectrum of the paste was recorded on a Perkin-Elmer Model 21 spectrophotometer equipped with sodium chloride optics.

The spectra are illustrated in Figures 1 through 11. Only the 7.0- to 15.0-micron portion is shown, as the spectra are almost identical in the functional group region between 2.0 and 7.5 microns. The absorption peak wave lengths are given in Table I.

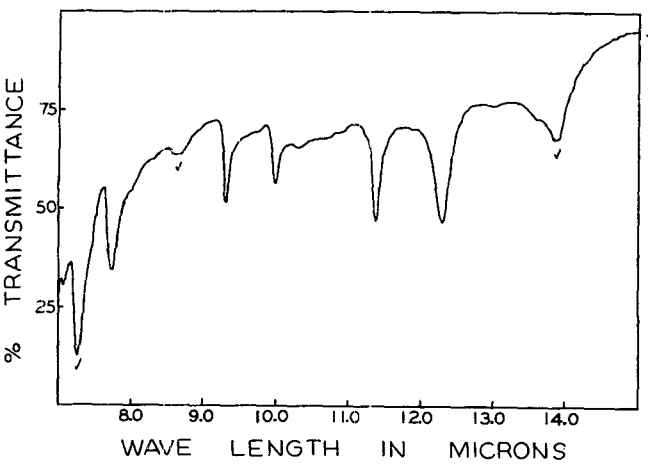


Figure 2. Sodium propionate

√. Mineral oil bands

Table I. Absorption Peak Wave Lengths of Sodium Salts of Some Mono- and Dibasic Acids

Salt	Wave Length, Microns		
Sodium acetate	9.55	9.95	10.84
Sodium propionate	7.73	9.30	9.99
	11.38	12.29	...
Sodium *n*-butyrate	7.45	7.98	9.16
	9.64	10.63	11.23
	11.36	13.40	14.35
Sodium *n*-valerate	7.05	7.62	8.11
	8.57	9.03	10.78
	11.22	11.90	12.45
	13.76	14.42	...
Sodium *n*-caproate	7.07	7.47	7.78
	8.20	9.07	9.95
	10.74	11.28	11.78
	14.42	...	...
Disodium oxalate	7.47	7.63	10.30
	12.90	...	...
Disodium malonate	7.94	8.48	10.30
	10.44	10.86	12.67
	14.25	...	...
Disodium succinate	8.20	8.54	10.83
	12.41	...	...
Disodium adipate	7.10	7.50	7.57
	8.33	8.81	10.90
	11.03	13.79	14.33
Disodium glutarate	7.04	7.64	8.11
	9.56	9.95	10.77
	11.45	11.98	13.17
Disodium pimelate	7.20	7.44	7.60
	7.93	8.35	9.16
	9.72	10.83	12.03
	14.24	14.75	...

QUANTITATIVE APPLICATION

The following method has been successfully applied to the quantitative determination of adipic acid in synthetic mixtures of adipic and glutaric acids and is given here as an example.

Sufficient sample was weighed into a 150-ml. beaker (tared with a stirring rod and a few glass beads) to provide approximately 5 grams of anhydrous sodium salts. After dilution to 20 ml. with water and addition of 1 drop of 1% phenolphthalein indicator solution, the mixture was titrated to a faint pink end point with 25% aqueous sodium hydroxide. If the presence of easily hydrolyzed esters is suspected, the solution should be held at 10° C. and titrated with weaker sodium hydroxide near the end point. In that event the titration is carried no further than the first definite end point.

The beaker was placed on a hot plate and the water was evaporated until only a thick slurry remained. This was cooled slightly, 125 ml. of acetone were added (while stirring to prevent lumping), and the salts were filtered through a tared, fritted-glass crucible. The beaker was rinsed with 150 ml. of acetone which were also passed through the filter crucible. (It is not necessary to remove all the salt from the beaker and stirring rod.) The crucible and salt were placed in the beaker containing the beads and stirring rod, and all were dried for 3 hours at 110° C., cooled, and weighed. The total weight of anhydrous salt was then calculated.

A 0.40-gram portion of the anhydrous salts and 2.0 ml. of mineral oil were mulled as in the preparation of samples for reference spectra. In the quantitative work, however, a 0.12-mm.,

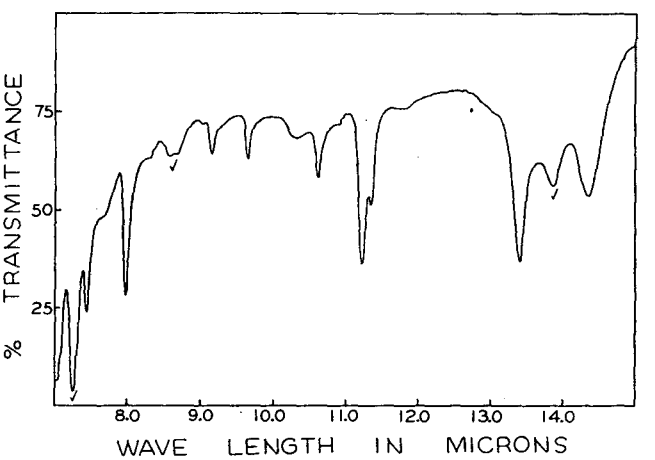


Figure 3. Sodium butyrate

√. Mineral oil bands

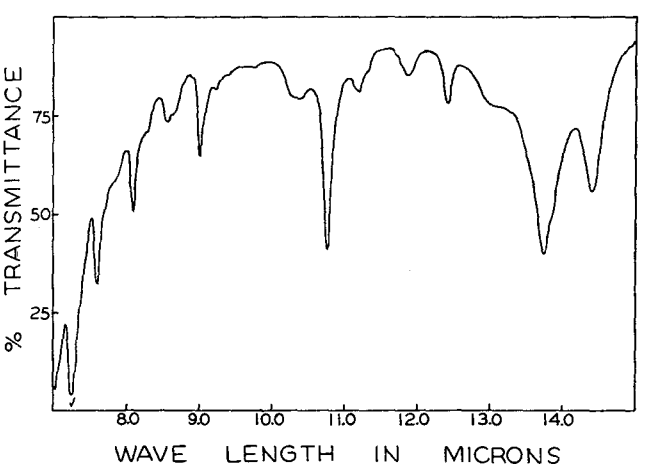


Figure 4. Sodium valerate

√. Mineral oil bands

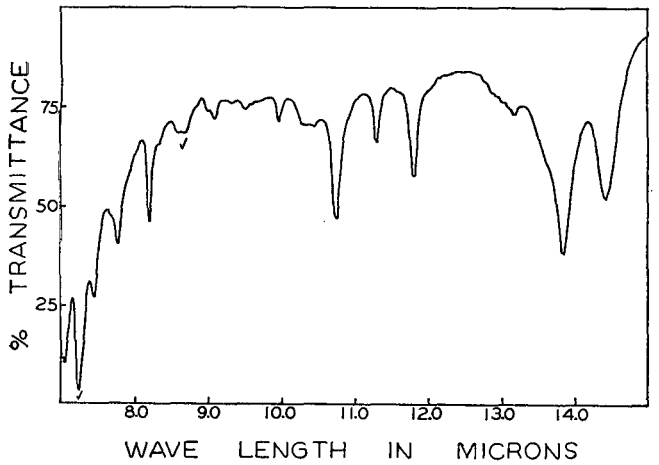


Figure 5. Sodium caproate

√. Mineral oil bands

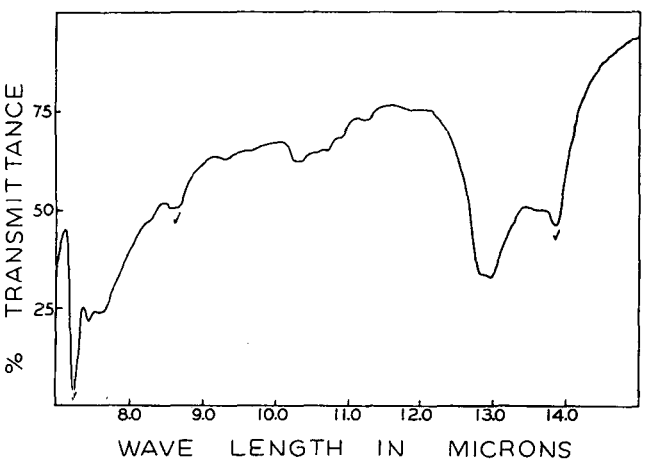


Figure 6. Disodium oxalate

√. Mineral oil bands

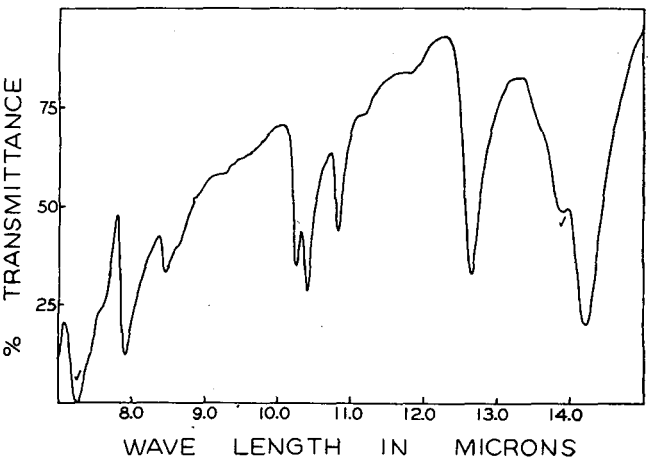


Figure 7. Disodium malonate

√. Mineral oil bands

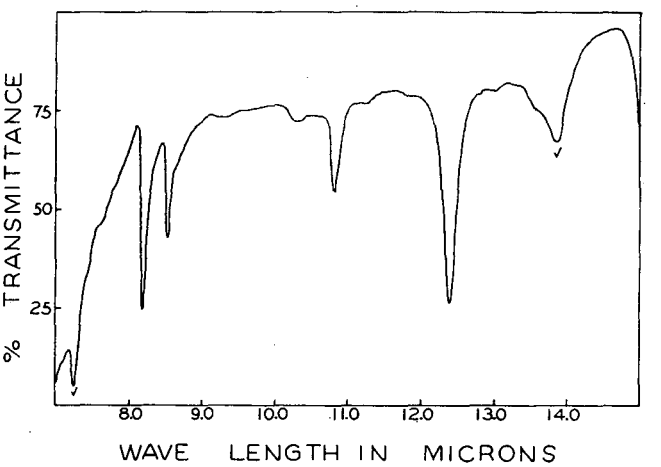


Figure 8. Disodium succinate

√. Mineral oil bands

instead of a 0.025-mm., slotted brass spacer was used to provide a higher absorbance. The mineral oil was prepared to contain 0.3% of pure naphthalene as an internal standard to serve as a reference for cell thickness, since the thickness of demountable cells cannot be exactly reproduced. Naphthalene was chosen specifically for the analysis described here and is not necessarily considered an ideal internal standard. The 12.8-micron naphthalene band is sufficiently intense so that no other naphthalene peaks appear in the spectra at the concentration level used.

A spectrum of the mull was recorded between 10.4 and 11.5 microns (disodium adipate analytical region) and between 12.3 and 13.5 microns (naphthalene region). The absorbance of the 11.03-micron disodium adipate band (A_1) and of the 12.8-micron naphthalene band (A_2) was measured using the standard base line technique. The ratio $\frac{A_1}{A_2}$ was then used to obtain the ratio $R = \left(\frac{\text{grams of disodium adipate}}{\text{grams of total salt}}\right)$ from a reference curve (Figure 12) prepared from standards of disodium adipate–disodium glutarate mixtures.

Calculation:

$$\% \text{ adipic acid} = \frac{R \times S \times 100}{W \times 1.301}$$

S = total weight of salt prepared, grams
W = acid sample weight, grams

1.301 = ratio of molecular weights $\frac{\text{disodium adipate}}{\text{adipic acid}}$

As the example given here exhibits a straight-line relationship (Figure 12) between R *vs.* A_1/A_2, the following expression may be used.

$$\% \text{ adipic acid} = \frac{[0.238\,(A_1/A_2) + 0.029] \times S \times 100}{W \times 1.301}$$

PREPARATION AND USE OF REFERENCE CURVE

A series of standards was prepared containing disodium adipate and disodium glutarate in varying proportions, the weight of disodium adipate per gram of salt being known. Infrared spectra of the mineral oil mulls of these salt mixtures were recorded, including the naphthalene peak for thickness reference. A curve was then prepared with A_1(disodium adipate)/A_2 (napthalene) plotted against grams of disodium adipate/grams of total salt. The same mineral oil mulling agent is used for standards and samples to correct for any variations in the preparation of the 0.3% naphthalene mulling agent.

Table II. Results of Determination of Adipic Acid in a Complex Mixture of Acids

Determination	Adipic Acid, %
1	11.4
2	11.4
3	11.3
4	10.9
5	11.1
6	10.3
7	11.3
8	10.5
9	11.6
10	10.8

PRECISION AND ACCURACY

This technique has been applied to the determination of adipic in a complex mixture containing glutaric, succinic, caproic, valeric, acetic, and ϵ-hydroxycaproic acids. The precision of the method is indicated by the results in Table II.

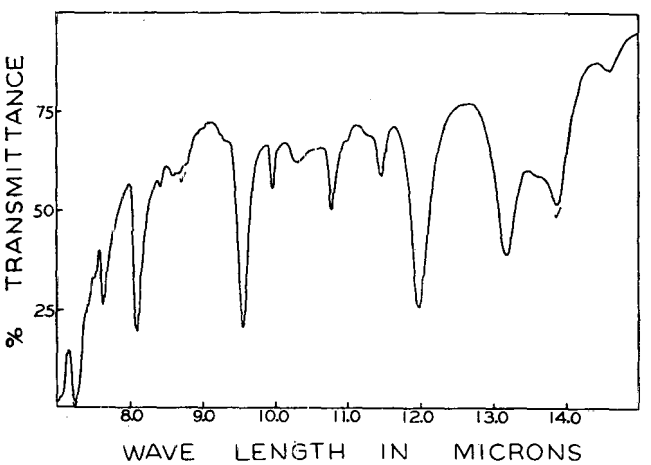


Figure 9. Disodium glutarate

√. Mineral oil bands

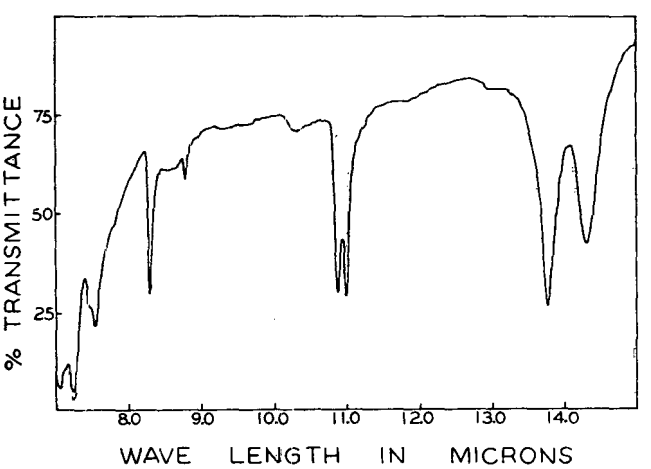


Figure 10. Disodium adipate

√. Mineral oil bands

This gives a standard deviation, σ, of 0.4% absolute or approximately 4% relative. A theoretical value of 3% relative should be obtained for duplicate determinations.

DISCUSSION

This procedure has been successfully applied to the analysis of complex mixtures of adipic and other organic acids where conventional methods of separation and analysis have been difficult and time-consuming. The time required for the complete analysis of a single sample is approximately 4 hours, including the drying time for the salt, but may be shortened considerably by using a vacuum dryer.

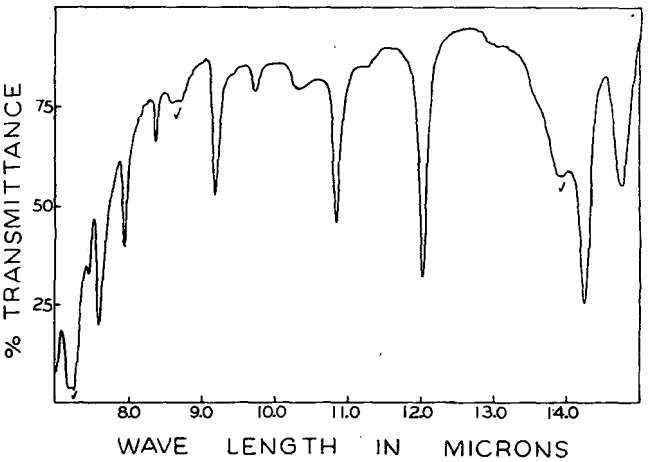


Figure 11. Disodium pimelate

Mineral oil bands

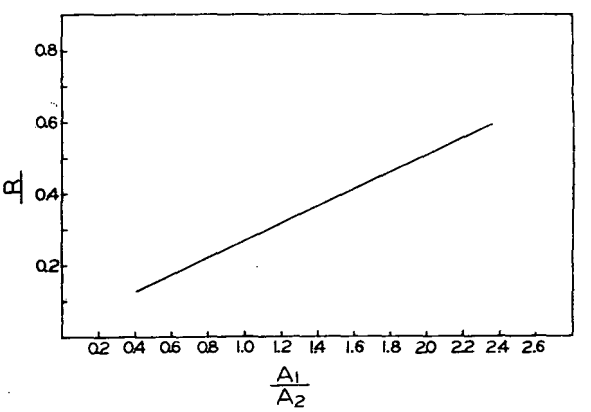


Figure 12. Reference curve

$$R = \frac{\text{weight of disodium adipate}}{\text{weight of total salt}}$$

$$\frac{A_1}{A_2} = \frac{\text{absorbance of disodium adipate at 11.03 microns}}{\text{absorbance of naphthalene at 12.8 microns}}$$

While the conventional mulling technique has proved entirely satisfactory in the majority of salts investigated by this laboratory, the new cold-pressing (potassium bromide pelleting) technique (*8*, *9*) should prove valuable in some cases. It is possible that greater sensitivity and precision can be obtained by the cold-pressing method, as it would eliminate the necessity of an internal standard for thickness reference. One potential drawback to the cold-pressing technique is the extreme hygroscopicity of some of the sodium salts. Salts of other metals might help overcome this difficulty.

The average particle size of the sodium salts of adipic and glutaric acids, obtained by using the mulling technique just described, is approximately 5 microns. Consequently, no significant variation in system linearity is experienced due to scattered radiation at the higher wave lengths used for the quantitative work (beyond 8 microns).

When the ratio grams of disodium adipate/grams of salt is used in the calculation, it must be remembered that not all salts will have the same specific gravity. Reasonably large variations

in the gravities of the salt diluents may be tolerated without appreciably affecting the results, as the volume of salt contributes little to the total volume of the mull.

D. M. Lewis of the Sabine River, Orange, Tex., plant has found the following alternative method satisfactory in analyzing adipic-glutaric acid mixtures. A sample containing about 2 to 4 grams of adipic acid is weighed into a 100-ml. volumetric flask, and neutralized to the phenolphthalein end point with 25% sodium hydroxide solution, and the contents of the flask are diluted to volume with water. After thorough mixing, a 10-ml. aliquot (pipet) of the solution is added, with vigorous stirring, to 250 ml. of acetone in a 600-ml. beaker. The precipitated disodium salts are filtered onto a tared, sintered-glass filter and dried to constant weight at 110° C. in a vacuum oven.

Exactly 0.200 gram of the dried salts is finely pulverized in a mortar, then mulled with 2.00 ml. of white mineral oil. A portion of the mull is transferred to a fixed 0.2-mm. disodium chloride cell. A second sodium chloride cell (about 0.05 mm. less in thickness) is filled with the white mineral oil. Both cells are placed in the Perkin-Elmer Model 21 infrared spectrophotometer (double-beam) and the sample is scanned between 10.4 and 11.5 microns. The absorbance at 11.03 microns is determined by the standard base line technique.

The concentration of disodium adipate is determined by comparison with the absorbance of a mull of a weighed amount of disodium adipate or by a calibration curve.

LITERATURE CITED

(1) Badger, R. M., and Bauer, S. H., *J. Chem. Phys.*, **5**, 605–8 (1937).
(2) *Ibid.*, pp. 369–70.
(3) Davies, M. M., and Sutherland, G. B. B. M., *Ibid.*, **6**, 755–70 (1938).
(4) Duval, C., Lecomte, J., and Douvillé, F., *Ann. phys.*, **17**, 5–71 (1942).
(5) Duval, C., Lecomte, J., and Douvillé, F., *Bull. soc. chim.*, **9**, 263–74 (1942).
(6) Duval, C., Lecomte, J., and Douvillé,, F., *Compt. rend.*, **212**, 953–6 (1941).
(7) Fichter, R., and Wehrli, M., *Helv. Phys. Acta*, **13**, 217–18 (1940).
(8) Schieldt, U., *Z. Naturforsch.*, **76**, 270 (1952).
(9) Stimson, M. M., *J. Am. Chem. Soc.*, **74**, 1805 (1952).
(10) Wehrli, M., and Schönmann, E., *Helv. Phys. Acta*, **15**, 317–19 (1942).

RECEIVED for review September 8, 1954. Accepted December 27, 1954.

Automatic Spectrophotometric Titration with Coulometrically Generated Titanous Ion

Determination of Vanadium in Titanium Tetrachloride

H. V. MALMSTADT and C. B. ROBERTS

Noyes Chemistry Laboratories, University of Illinois, Urbana, Ill.

This investigation was undertaken to find a simple method of sample preparation and of determining vanadium in titanium tetrachloride which could be carried out rapidly to give precise and accurate results. Titanium tetrachloride was hydrolyzed without loss of vanadium by pipetting a sample into a hydrochloric acid solution in a flask fitted with a reflux condenser and water trap. The vanadium in the hydrolyzed titanium sample was determined directly by a rapid and accurate titrimetric procedure using automatic spectrophotometric end point detection and coulometric generation of titanous ion. Ten-milliliter samples containing about 0.01 to 0.2% of vanadium in titanium tetrachloride were determined with an average deviation of about 0.00016%. The results indicate the possible general usefulness of coulometric generation of titrant for automatic spectrophotometric titrations, and the specific application of the method for the rapid and accurate titrimetric determination of vanadium in titanium tetrachloride.

THE importance of titanium tetrachloride as an intermediary in the manufacture of titanium sponge has led to its analysis for impurities. Vanadium is a principal impurity in titanium tetrachloride, and it is important to have a rapid and accurate method for its determination. A method of sample preparation was first developed so that the titanium tetrachloride sample could be rapidly hydrolyzed without loss of sample. The hydrolysis was performed by adding the sample to a hydrochloric acid solution in a flask immersed in an ice bath and fitted with a reflux condenser topped with a water trap. The resulting solution of titanium and its impurities was about 7.5*M* in hydrochloric acid, and contained the vanadium as both vanadium(IV) and vanadium(V).

Goddu and Hume (*2*) developed a good photometric titration procedure for the determination of vanadium in steel, but the permanganate titration was not directly applicable to the immediate titration of the strong hydrochloric acid solutions of hydrolyzed titanium tetrachloride. Arthur and Donahue (*1*) used coulometrically generated titanous ion for the titrimetric determination of iron, and it appeared that this procedure would be expedient for the determination of vanadium in titanium tetrachloride because the hydrolyzed sample might be directly analyzed without external addition of reagents. Since the titanium tetrachloride and its hydrolyzed solution contained the vanadium as both vanadium(V) and vanadium(IV), it was necessary either to oxidize all the vanadium to vanadium(V), which did not prove successful in strong hydrochloric acid, or to determine the amount of titanous ion used in going from the first end point [vanadium(V) to vanadium(IV)] to the second end point [vanadium(IV) to vanadium(III)].

Potentiometric determination (*3*) of the end points in the hydrochloric acid solution was not too successful because the second end point was not sharp and was impossible to determine accurately. An investigation of spectrophotometric detection of the end points yielded precise and accurate results for the determination of vanadium in titanium tetrachloride and indicated the possible general usefulness of coulometric generation of titrant for automatic spectrophotometric titrations.

Several excellent spectrophotometric titration procedures have been developed within the past few years. Sweetser and Bricker (*10, 11*) used the Beckman Models DU and B for manual spectrophotometric titrations in both the visible and ultraviolet spectral regions. Reilley and Schweizer (*6*) have extended its use to nonaqueous solvents, and Underwood (*12, 13*) has used a similar procedure to determine iron, copper, and bismuth. Malmstadt and Gohrbandt (*4*) used a Cary spectrophotometer for automatic spectrophotometric titrations to determine thorium. In all of these procedures the titrant was a standard solution which was added by a buret. In the present work no standard solution

was used; titanous ion was generated electrolytically, the number of equivalents added being calculated from measurements of current and time. Wise, Gilles, and Reynolds (*14*) developed an automatic coulometric titration instrument which furnished constant current and automatically stopped the titration by means of photometric detection of the end point but their method depended on a large color change at the end point and did not give a continuous recording of color change. The procedure described herein has the advantage of permitting extrapolation to an end point when there is only a slight change of absorbance at the equivalence point because of dilute solutions or the nature of the color change itself.

Because the Cary spectrophotometer can automatically record time versus absorbance, it is necessary to have only a suitable titration cell, a motor-driven stirrer, a constant current source, and a means by which to measure the current. By using Faraday's law, the number of equivalents of titrant added can be calculated at any time. Thus a recorded plot of absorbance *vs.* equivalents is easily obtained.

This method has all the advantages which have been mentioned in previous literature on spectrophotometric titrations (*2, 4, 6*). In addition, it has no dilution error, as the volume remains constant during the titration. The same precautions for coulometric titrations, such as 100% electrode efficiency for the titrant and rate of attaining equilibrium, must be observed.

Arthur and Donahue (*1*) used a gold-plated platinum electrode in approximately 0.7*M* hydrochloric acid as the generator cathode for their titration of iron with titanous ion. They experienced difficulty in cleaning and maintaining their electrode so that it would not evolve hydrogen. In the present investigation it was possible to use a titanium metal rod as the cathode in about 1 to 12*M* hydrochloric acid solutions with 100% current efficiency in generating titanous ion and no significant attack on the titanium metal was observed. Platinum was also used as the cathode with 100% efficiency for generation of titanous ion in hydrochloric acid solutions greater than 7*M*, but hydrogen was evolved at lower acid concentrations. In all cases with both electrodes the current did not exceed 100 ma.

Samples containing about 0.01 to 0.2% vanadium in titanium tetrachloride were determined with an average deviation of about 0.00016%. The possible interference of iron in the redox titration of vanadium by titanous ion was studied and it was found possible to determine the two simultaneously.

APPARATUS

The Cary spectrophotometer titration vessel was essentially the same as used by Malmstadt and Gohrbandt (*4*) in their determination of thorium. The only changes made were in the height of the cell and the cover for the cell compartment. The cell was increased in height until it touched the bottom of the cover through which a 2-inch hole was cut. A No. 11 stopper was fitted into this so that it formed a tight seal with both cover and cell. In this way hydrochloric acid fumes were kept from the inside of the instrument and light was excluded from the cell compartment. Three holes were bored in the stopper to permit passage of the stirrer, cathode, and anode.

Coulometric Electrodes. Two different cathodes were used, and both gave the same results in strong hydrochloric acid solutions. One cathode consisted of a piece of 75A titanium rod, 4 mm. in diameter and 13 cm. long. The rod extended into the titration cell so that approximately 0.5 inch was in contact with the solution. The other cathode was a square platinum foil connected to a platinum wire sealed in a glass tube.

The anode was a piece of platinum foil 1.5 by 1.5 cm. It was isolated from the solution that was being titrated by inserting it into a glass tube 1.0 cm. in diameter and 10 cm. long, with a medium porosity sintered-glass disk sealed at the bottom end. The tube was filled to a height of about 1 inch with 1*N* sulfuric acid, and the platinum electrode was immersed in this. The part of the tube extending above the stopper was painted black in order to exclude light from the titration cell compartment.

Stirrer. The stirrer was the same as that used by Malmstadt and Gohrbandt (*4*). It passed through the center of the stopper with the coulometric electrodes on either side.

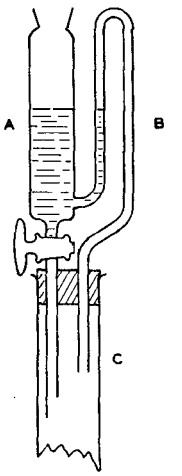


Figure 1. Top of condenser and water trap for hydrolysis

A. 60-ml. separatory funnel
B. Side arm
C. Top of reflux condenser

Constant Current Source. Constant current was provided by a circuit similar to that described by Reilley, Cooke, and Furman (*5*).

External Circuit. The external circuit was the same as that shown by Reilley and coworkers (*5*) for macrotitrations, except that a 110-volt, double-pole, double-throw relay operated in place of the double-pole, double-throw shorting type switch. The relay and a Time It stop clock were connected through a single-pole, single-throw switch to the 110-line voltage, and a dummy resistor was used when the cell was not in operation. The voltage drop across a 3.513-ohm standard resistor was measured by a Leeds and Northrup potentiometer.

Apparatus for Hydrolysis of Titanium Tetrachloride. A 24-inch water-cooled reflux condenser was fitted with a rubber stopper so that it could be easily attached and removed from one of the side holes of a 500-ml., three-hole, round-bottomed flask. A solid rubber stopper was placed in the larger center hole, and the other side hole was fitted with a 1-hole, No. 3 stopper through which passed a 10-ml. pipet. A 2-hole rubber stopper was placed in the top of the reflux condenser (see Figure 1), and a 60-ml., straight-sided, borosilicate glass separatory funnel and a side arm of 6-mm. diameter glass tubing were fitted into the stopper. The loop in the side arm was made approximately the same height as the top of the separatory funnel. In this way all volatile materials which did not condense in the reflux condenser bubbled through the water in the separatory funnel. The water was used to wash the condenser and bring the reaction mixture in the flask to the correct acid concentration for the titration.

PROCEDURE AND EXPERIMENTAL RESULTS

Preparation of Sample. Samples hydrolyzed in the open did not give consistent results for vanadium from one sample to another. Therefore, the following method was used to prepare samples so that all the vanadium was retained and gave a correct acid concentration.

Approximately 60 ml. of concentrated hydrochloric acid (12*M*) were placed in the round-bottomed flask, the flask was attached to the condenser, and 40 ml. of distilled water were placed in the separatory funnel. Ice was placed in a pan under the flask to remove some of the heat of hydrolysis. A 10-ml. sample of titanium tetrachloride was pipetted slowly into the flask through one side arm. The stopper on the pipet was immediately put in place to prevent the loss of volatile material. Most of the hydrogen chloride and volatile impurities were either condensed in the reflux condenser or dissolved in the water in the water trap, although a small amount of material in the form of white fumes passed through the water. The fumes were not determined because the accuracy and reproducibility of the results showed that no vanadium passed through the combination of reflux condenser and water trap.

After all the titanium tetrachloride was added from the pipet, the finger was held tightly over the end of the pipet and the stopcock of the separatory funnel was opened momentarily. The presence of water in the condenser caused a partial vacuum by dissolving hydrogen chloride gas. This caused a rapid removal of water from the separatory funnel through the side arm and washed the condenser free of all condensate. Simultaneously with this operation, the finger was removed from the pipet and all of its remaining contents were drawn into the reaction flask. The stopcock on the separatory funnel was then opened and the remaining water was allowed to drain into the reaction flask. The solution was transferred directly to the titration vessel, and the reaction flask was washed with 10 ml. of concentrated hydrochloric acid which were added to the solution in the titration vessel.

A sample prepared in this manner was brown in color and contained a small amount of chlorine and oxygen which gave high results if not removed. This was done by covering the titration cell with a Teflon shield to prevent possible loss of material, and bubbling tank carbon dioxide through the solution vigorously for about 10 minutes.

Spectrophotometric End Point Detection. The reduction of vanadium by titanous ion takes place in two steps. Vanadium which is present in the vanadium(V) oxidation state is first reduced to vanadium(IV) and this in turn is reduced to vanadium(III). Reduction of vanadium stops at this point and purple titanous chloride appears. The presence of vanadium(IV) in the hydrolyzed titanium tetrachloride solutions was indicated by the smaller number of equivalents of titanous ion required to reach the first end point than between first and second end points. Attempts were made to oxidize all the vanadium to vanadium(V) so that only one end point would be necessary, but these failed because of the high concentration of chloride ion. An accurate method resulted when the time of reduction for vanadium was taken as the difference between the vanadium(V)–vanadium(IV) end point and the vanadium(IV)–vanadium(III) end point, and did not require any addition of reagents prior to titration.

One wave length which was used successfully for the titration was 490 mμ. This was decided upon after scanning the solution at different stages of electrolysis. The vanadium(V) complexes with chloride in strong hydrochloric acid solutions to give strong absorption of light in the region of 490 mμ. Figure 2, *A*, shows a typical titration curve which was obtained at this wave length. The current in this and all other titrations was approximately 57 ma., and was determined accurately for each run.

The acid concentration is rather critical when the titration is performed at a wave length of 490 mμ, because it determines the slope of the vanadium(V)–vanadium(IV) absorption line. If the acid concentration is too great the line is very steep and the pen is off scale except in the region of the end point; if the concentration is too low the slope is too small and the end point is more difficult to locate. Best results were obtained when 10 ml. of titanium tetrachloride were hydrolyzed in the equivalent of 110 ml. of 7.5*M* hydrochloric acid. Acid and water can be measured accurately enough in a graduated cylinder. The quantities given in the procedure for hydrolysis will give this concentration.

A wave length of 760 mμ, which Sabatini, Hazel, and McNabb (*7*) determined as the maximum for vanadyl ion, was also used to determine the spectrophotometric end point. The titration curve at this wave length is shown in Figure 2, *B*.

The results obtained at 490 and 760 mμ are comparable in precision and accuracy. When only a small amount of vanadium(V) is present a sharper first end point is obtained at a wave length of 490 mμ.

It was found that equilibrium was quickly attained whenever the electrolysis was stopped at intervals during the titration. By using the stop clock and plotting absorbance against time, the same results were obtained as when the titration was automatically recorded.

The stoichiometry of the method was tested with known quantities of vanadium. Known amounts of 99.97% pure vanadium pentoxide (V_2O_5) were dissolved in concentrated hydrochloric acid and added to the reaction flask. Ten-milliliter aliquots of purified Crane titanium tetrachloride were then added to the flask and the acid was brought to the proper strength as previously described.

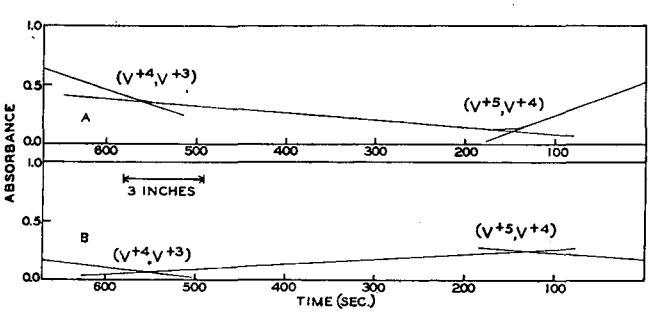


Figure 2. Titration of vanadium with coulometrically generated titanous ion

A. Wave length 490 mμ
B. Wave length 760 mμ

The results for the spectrophotometric titration of known amounts of vanadium are shown in Table I. Determinations 1 to 6, inclusive, were run at a wave length of 760 mμ, and determinations 7, 8, and 9 were run at a wave length of 490 mμ. The average deviation for nine runs was 0.03 mg.

Table I. Spectrophotometric Titration of Known Amounts of Vanadium

Run No.	Mg. of V Taken	Mg. of V Found	Difference, Mg.
1	4.06	4.06	±0.00
2	4.06	4.09	+0.03
3	4.06	4.12	+0.06
4	8.01	8.02	+0.01
5	8.01	8.04	+0.03
6	39.56	39.42	−0.14
7	39.56	39.58	+0.02
8	39.56	39.55	−0.01
9	39.56	39.57	+0.01

The total amount of vanadium taken was the sum of the vanadium in the vanadium pentoxide and the small amount contained in the purified Crane titanium tetrachloride. Spectrographic analysis showed the purified titanium tetrachloride to contain about 0.0005% of vanadium. This was determined more accurately by taking different known amounts of vanadium pentoxide and determining by titration the total quantity of vanadium present. It was calculated by difference that the Crane purified contained 0.0006% vanadium, or 0.12 mg. per 10 ml. of titanium tetrachloride.

Table II. Determination of Vanadium in Titanium Tetrachloride Samples

Run	Source	Calcd. Vanadium, %	Vanadium Found, %	Deviation
1	Synthetic	0.2292	0.2293	+0.0001
2	Synthetic	0.2292	0.2291	−0.0001
3	Synthetic	0.2292	0.2284	−0.0008
4	Synthetic	0.2292	0.2293	+0.0001
5	Crane Co. crude	....	0.1147	....
6	Crane Co. crude	....	0.1150	....
7	Fisher technical	....	0.0896	....
8	Fisher technical	....	0.0898	....
9–15	Cenco technical	....	0.0731[a]	....
16	Cenco technical, 5 ml. Crane pur., 5 ml.	0.0366	0.0367	+0.0001
17	Cenco technical, 5 ml. Crane pur., 5 ml.	0.0366	0.0364	−0.0002
18	Cenco technical, 2 ml. Crane pur., 10 ml.	0.0127	0.0124	−0.0003
19	Cenco technical, 2 ml. Crane pur., 10 ml.	0.0127	0.0124	−0.0003

[a] Average value, average deviation = 0.00016%.

Analyses for vanadium were made on the following commercial sources of titanium tetrachloride: Crane Co. crude, Fisher technical grade, Cenco technical grade, and mixtures of different proportions of Cenco technical grade and Crane purified.

Table II shows these results together with results on four synthetic samples of high vanadium content. These were the same samples as 6 to 9 in Table I, but the calculations were presented as percentage of vanadium in titanium tetrachloride. The amount of vanadium in runs 16 to 19 was calculated by averaging runs 9 to 15, inclusive, calculating the amount of vanadium in the volume of Cenco technical titanium used, and correcting for residual vanadium. The weight of the titanium tetrachloride sample was determined for each 10-ml. aliquot by calculating the specific volume according to the formula given by Sagawa (*8*, *9*):

$$V_t = 0.56773\,(1 + 9.645 \times 10^{-4}t + 6.026 \times 10^{-7}t^2 + 5.94 \times 10^{-9}t^3)$$

where t is temperature in degrees Centigrade. This was found to be accurate within the experimental limits of the method.

INTERFERENCES

The only interference studied was that of iron, as it was considered the most probable impurity besides vanadium. No iron was found in the commercial samples of titanium tetrachloride, so synthetic samples were prepared by adding a standard solution of ferric chloride to a hydrolyzed solution of Cenco technical titanium tetrachloride. Acid strengths were the same as those used for the determination of vanadium alone.

Table III. Determination of Iron and Vanadium in Titanium Tetrachloride

Run	Source	Iron, Mg. Calcd.	Iron, Mg. Found	Vanadium, Mg. Calcd.	Vanadium, Mg. Found
1	2 ml. 0.0513*M* $FeCl_3$ 10 ml. Cenco tech. $TiCl_4$	5.73	5.7	12.62	12.6
2	4 ml. 0.0196*M* $FeCl_3$ 10 ml. Cenco tech. $TiCl_4$	4.38	4.3	12.62	12.8
3	4 ml. 0.0196*M* $FeCl_3$ 10 ml. Cenco tech. $TiCl_4$	4.38	4.3	12.62	12.8

Figure 3, *A*, shows the titration curve at a wave length of 490 mμ, and Figure 3, *B*, shows the titration curve at 760 mμ. The presence of iron causes a flattening of the vanadium(IV)–vanadium(III) absorbance line near the second end point, thereby making the end point more difficult to determine. By using the straight portion of the lines near the vanadium(IV)–vanadium (III) and iron(III)–iron(II) end points, fairly accurate results for both elements can be obtained although they are not so accurate as for vanadium alone. A possible improvement could be made by adding a reagent which would form a ferrous complex of high absorbance.

Table III shows the results for analyses of per cent vanadium and iron in the same titanium tetrachloride solution. Titrations 1 and 2 were made at 490 mμ and titration 3 was made at 760 mμ. The iron solutions were standardized with potassium dichromate.

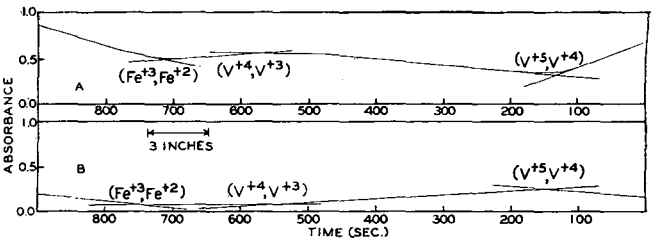


Figure 3. Simultaneous determination of iron and vanadium in titanium tetrachloride

A. Wave length 490 mμ
B. Wave length 760 mμ

ACKNOWLEDGMENT

The authors are indebted to Cramet, Inc., Chattanooga, Tenn., for financial support of this project.

LITERATURE CITED

(1) Arthur, P., and Donahue, J. F., ANAL. CHEM., **24**, 1612 (1952).
(2) Goddu, R. F., and Hume, D. N., *Ibid.*, **22**, 1314 (1950).
(3) Kolthoff, I. M., and Furman, N. H., "Potentiometric Titrations," 2nd ed., p. 373, Wiley, New York, 1931.
(4) Malmstadt, H. V., and Gohrbandt, E. C., ANAL. CHEM., **26**, 442 (1954).
(5) Reilley, C. N., Cooke, W. D., and Furman, N. H., *Ibid.*, **23**, 1030 (1951).
(6) Reilley, C. N., and Schweizer, B., *Ibid.*, **26**, 1124 (1954).
(7) Sabatini, R., Hazel, J., and McNabb, W., *Anal. Chim. Acta*, **6**, 368 (1952).
(8) Sagawa, T., *Complete Abstr. Japan. Chem. Lit.*, **6**, 341 (1932).
(9) Shirrer, G., Johnston, H. L., and Beckett, C., "Titanium and Its Compounds," p. 43, Herrick L. Johnston Enterprises, Columbus, Ohio, 1954.
(10) Sweetser, P. B., and Bricker, C. E., ANAL. CHEM., **24**, 409 (1952).
(11) *Ibid.*, **25**, 253 (1953).
(12) Underwood, A. L., *Ibid.*, **25**, 1910 (1953).
(13) *Ibid.*, **26**, 1322 (1954).
(14) Wise, E. N., Gilles, P. W., and Reynolds, C. A., Jr., *Ibid.*, **25**, 1344 (1953).

RECEIVED for review September 20, 1954. Accepted December 30, 1954.

Determination of Erythromycin by Ultraviolet Spectrophotometry

JOHN B. TEPE and C. V. ST. JOHN
Eli Lilly & Co., Indianapolis, Ind.

The development of an accurate method for the determination of erythromycin was based on the observation that alkaline hydrolysis produced a material that had a characteristic ultraviolet absorption. An acid-inactivated blank was employed to correct for the ultraviolet absorbing impurities and degradation products of erythromycin. The assay has shown good reproducibility, sensitivity, and correlation with a turbidimetric microbiological assay.

ERYTHROMYCIN (*7*) is a basic antibiotic that is used extensively for the treatment of various bacterial infections. An accurate method of assay was desired for processing and formulation, during the manufacturing of this antibiotic. A colorimetric assay for erythromycin reported by Ford and coworkers (*6*) employed sulfuric acid to develop a color that absorbed at 485 mμ. Acid-hydrolyzed erythromycin (*8*) reacted with Nelson's reagent to develop a blue color. These methods do not appear to be specific for erythromycin.

During the course of this investigation for a chemical method of analysis, many color reactions were studied. Those that gave positive reactions with pure erythromycin are listed with comments as to their usefulness in analyzing samples of varying purities that are encountered in the fermentation and purification of the antibiotic. The procedures and conditions employed were the same as described in the literature.

1. Erythromycin when heated with a mixture of glacial acetic acid and perchloric acid (varying proportions) gave a color that absorbed strongly at 485 mμ. Reproducibility was good but the test gave higher values on process samples than microbiological assay or the ultraviolet assay.
2. Erythromycin was oxidized by periodic acid and the resultant formaldehyde was determined by chromotropic acid (*2*). The reproducibility was good but the test was not sufficiently sensitive for dilute solutions.
3. The tryptophan–perchloric acid reagent (*3*) reacted with erythromycin to produce a red color. This method had good sensitivity and reproducibility; however, degradation products of erythromycin in impure samples reacted with the reagent.
4. The xanthydrol reagent (*9*) reacted with the erythromycin to give a red color that absorbed strongly at 540 mμ. The sensi-

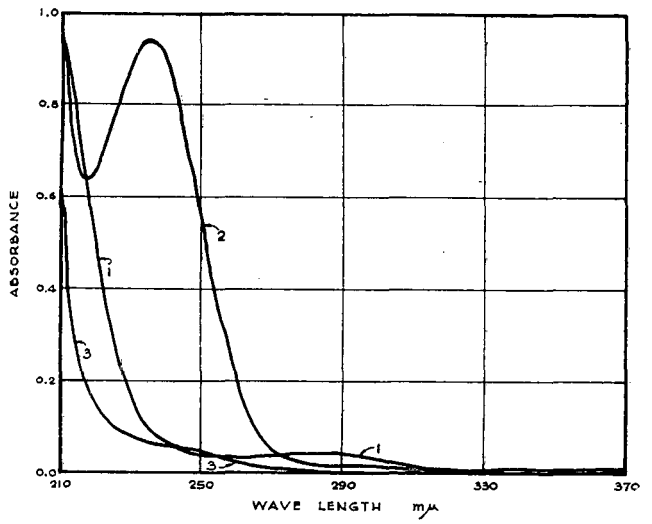


Figure 1. Absorption spectra of erythromycin

1. Aqueous solution, 1000 γ per ml.
2. Five-milliliter sample containing 1000 γ, hydrolyzed with 1.0 ml. of 0.05*N* NaOH, at 100° C., 5 minutes, final dilution 100 γ per ml.
3. Acid-inactivated blank, 100 γ per ml.

tivity and reproducibility were good; however, degradation products from erythromycin caused variable results.

Other reactions were investigated and found useful for the analysis of pure samples but were not applicable for process samples because of a lack of sensitivity or specificity. The reactions studied included the treatment of erythromycin with *p*-hydroxybiphenyl (*1*), Folin-Ciocalteau phenol reagent (*5*), diphenylamine in acetic acid and sulfuric acid (*4*), phosphomolybdic acid (*12*), and diazotized sulfanilic acid and sodium hydroxide (*11*).

After considering the above procedures, it was found that the characteristic ultraviolet absorption properties of hydrolyzed erythromycin offered the most promising method for analysis. Erythromycin exhibits a broad weak absorption band (Figure 1) in the ultraviolet at 285 mμ. After strong acid hydrolysis at elevated temperatures (Figure 2), erythromycin exhibits maxima at 226, 267, and 485 mμ. The absorption at 226 mμ has an $E^{1\%}_{1\ cm.}$ of approximately 150 and obeys Beer's law. Other degradation products of erythromycin also have absorption in the 226-mμ range and limit the usefulness of acid hydrolysis as an assay method. After dilute alkaline hydrolysis, erythromycin exhibits strong absorption at 236 mμ, with an $E^{1\%}_{1\ cm.}$ of 85. A dilute acid-inactivated blank can be used to correct for the ultraviolet absorption of degradation products and impurities. An untreated blank is not satisfactory because of the changes that may occur in impurities during the alkaline hydrolysis.

This procedure has been employed for assaying erythromycin concentrates after separation from the fermentation broth. It has shown very good correlation with microbiological assay on processing and development samples.

APPARATUS

A Beckman Model DU quartz spectrophotometer equipped with a photomultiplier tube and ultraviolet radiation source was used for all measurements other than the absorption curves shown. The curves were obtained on a Cary Model 12 recording spectrophotometer. One-centimeter quartz cells and water blanks were used for all measurements.

REAGENTS

Sodium hydroxide, 0.05*N* aqueous solution, prepared from a standardized 1.00*N* solution.

Sulfuric acid, 0.05*N* aqueous solution, prepared from a standardized 1.00*N* solution.

Diglycolic acid (Matheson, Coleman and Bell, 5973), 0.1% aqueous solution, with pH adjusted to 4.0 with 1*N* sodium hydroxide.

RECOMMENDED PROCEDURE

Preparation of Standards. Weigh accurately an amount of erythromycin primary standard equivalent to 25 mg. of erythromycin base and transfer to a 25-ml. volumetric flask. Add 1.0 ml. of absolute methanol to dissolve the erythromycin and dilute to the mark with freshly distilled water. Prepare this solution immediately before using. Dilute aliquots of this solution with freshly distilled water, so that 5.0-ml. aliquots will contain 100, 200, 300, 400, 500, 600, 700, and 800 γ of erythromycin activity.

Standardization. Transfer 5.0-ml. aliquots of the diluted standards to four 10-ml. volumetric flasks. To two of these (the blanks) add 0.5 ml. of the 0.05*N* sulfuric acid. Allow the blanks to react at room temperature (25° C.) for 1 hour. To the other flasks (the samples) add 1.0 ml. of 0.05*N* sodium hydroxide and immediately heat 5 minutes in an actively boiling water bath. Cool, dilute to mark, and read absorbance against distilled water at 236 mμ within 20 minutes. After the blanks have reacted for 1 hour, add 1.5 ml. of 0.05*N* sodium hydroxide solution, heat in actively boiling water for 5 minutes, and treat in the same manner as the sample. The difference in absorbance between the blanks and samples of respective concentrations is used to establish the standard curve.

Preparation of Sample. Samples of Dry Material. Weigh and dissolve samples of erythromycin base or its salts as outlined for standard. Dilute so that a 5.0-ml. aliquot will contain approximately 500 γ of activity.

Samples of Aqueous Solutions. Make appropriate dilutions so that a 5.0-ml. aliquot will contain approximately 500 γ of activity.

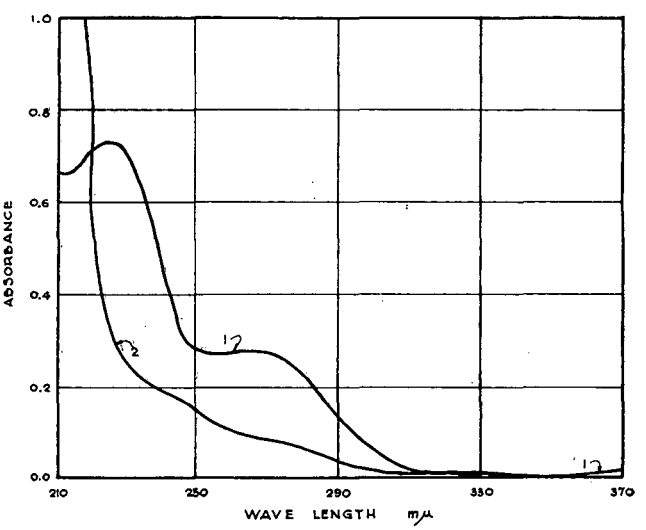


Figure 2. Absorption spectra of hydrolyzed erythromycin

1. Five-milliliter sample containing 1000 γ, hydrolyzed with 1.0 ml. of 1.0*N* H_2SO_4, 30 minutes, 100° C., final dilution 100 γ per ml.
2. Five-milliliter sample containing 1000 γ, hydrolyzed with 1.0 ml. of 1.0*N* NaOH, 30 minutes, 100° C., final dilution 100 γ per ml.

Nonaqueous Solutions. Evaporate an appropriate amount to dryness, dissolve the residue in 1 or 2 ml. of methanol, and dilute with water so that a 5.0-ml. aliquot will contain approximately 500 γ of erythromycin. As an alternative, the erythromycin may be extracted from water-immiscible solvents with a 0.1% aqueous solution of diglycolic acid at pH 4.0. Carry out the extraction in centrifuge tubes at ice bath temperature, and at a ratio that will give an aqueous phase having a pH of approximately 5. Dilute the aqueous phase as previously described. The extraction ratio is determined experimentally and is dependent upon the concentration and purity of the erythromycin in the nonaqueous phase. In most cases, a ratio of 5 parts of organic phase to 3 parts of diglycolic acid solution will satisfy the above conditions. Recovery experiments with pure erythromycin dissolved in amyl acetate at varying concentrations give recoveries of 94 to 96% with the single extraction.

Table I. Effect of Duration of Heating Period, and Amount and Concentration of Sodium Hydroxide on Absorption of Erythromycin

	Concentration of Sodium Hydroxide			
	0.05*N*	0.05*N*	0.05*N*	0.10*N*
	Sodium Hydroxide, Ml.			
	0.9	1.0	1.1	1.0
Duration of Heating Period, Minutes	Absorbance at 236 mμ			
1		0.096		0.216
1.5				0.245
2		0.262		0.237
3		0.383		0.212
4		0.428		0.203
5	0.430	0.436	0.436	0.192
6		0.436		
7		0.438		
8		0.433		
10		0.429		

EXPERIMENTAL

Conditions for Hydrolysis. The spectra obtained under different conditions of alkaline hydrolysis are shown in curve 2 (Figure 1) and curve 2 (Figure 2). In general, too high an alkali concentration or prolonged heating was found to result in reduced absorption at 236 mμ. It was necessary to balance the time and temperature of heating with the concentration of alkali in order to obtain maximum sensitivity. For convenience, a temperature of 100° C. was selected for hydrolysis. Time and pH were then determined for maximum sensitivity. It was found that a pH of approximately 12 was necessary for complete hydrolysis. Below this pH, hydrolysis of solutions of pure samples was observed, but with process samples containing other material, hydrolysis was not complete. Table I shows the effect of sodium hydroxide concentration and time of heating at 100° C. on absorption of erythromycin solutions. These data show that heating with 0.05*N* sodium hydroxide for 5 minutes gives maximum absorbance. Table I also shows the effect of a 10% variation in the specified 1.0 ml. of 0.05*N* sodium hydroxide.

Sample Blank. After hydrolysis at a pH of 2 to 3, the erythromycin does not give an absorbance maximum at 236 mμ when hydrolyzed with alkali (Figure 1). The absorbance of the alkaline-hydrolyzed, acid-treated blank decreased rapidly as shown in Table II. A time of 60 minutes was chosen since the reaction is complete at that time. By the use of this blank, it was possible to correct for the ultraviolet absorption of benzoate salts and penicillin when these are components of formulations containing erythromycin.

Reproducibility. Table III lists the absorbance of eight sets of duplicate determinations, and an evaluation of these results.

Table II. Absorbance of Caustic-Hydrolyzed Blank after Acid Treatment[a]

Time of Acid Inactivation, Minutes	Absorbance at 236 mμ	Time of Acid Inactivation, Minutes	Absorbance at 236 mμ
10	0.089	50	0.033
20	0.046	60	0.030
30	0.036	70	0.030
40	0.036	90	0.030

[a] **Conditions.** 5.0-ml. aliquot treated with 0.5 ml. of 0.05*N* sulfuric acid for time indicated. Sample then treated with 1.5 ml. of 0.05*N* sodium hydroxide and heated for 5 minutes at 100° C. Erythromycin concentration was 50 γ per ml.

Table III. Reproducibility of Ultraviolet Assay

Sample	Absorbance	Sample	Absorbance
1	0.407–0.409	5	0.392–0.391
2	0.406–0.405	6	0.403–0.401
3	0.406–0.403	7	0.412–0.413
4	0.420–0.418	8	0.405–0.402
Average	0.406	Average deviation	0.0056
Mean	0.405	Mean deviation	0.0056

Standard deviation = $\sqrt{\frac{\Sigma (X - \bar{X}^2)}{N}}$ = 0.0076.

These analyses were on different weights of erythromycin, but were corrected to unit weight in the table (50 γ per ml).

Sensitivity. The absorbance of the alkaline-hydrolyzed erythromycin follows Beer's law from concentrations of 10 γ per ml. to 100 γ per ml. of activity. The molar absorbance coefficient is about 5800.

Interferences. The small amount of solvents present after evaporation or extraction of the samples did not affect the test. Methanol, which has no effect up to a concentration of 1% of final dilution, was used as a solvent whenever needed. One per cent of acetone in the final dilution results in an assay that is 3 to 5% low.

This procedure has been used for 18 months for analysis of samples from fermentation processes. No serious interfering agents have been encountered in samples obtained at various stages of purification of the antibiotic. Erythromycin B (*10*), which has an activity of less than 60 γ per mg. when assayed by this procedure, is not a serious interference, as it is normally present in small amounts in samples taken from this process.

DISCUSSION

The described procedure has been employed for determining the potency of fermentation samples. The erythromycin is extracted from the broth at pH 10 with amyl acetate or trichloroethylene, after which the procedure for nonaqueous samples is used. It was necessary to restandardize the test on the basis of recoveries of erythromycin through this extraction procedure. Recoveries through this procedure varied from 90 to 94%.

Good agreement has been obtained on a large number of process samples that have been assayed by the ultraviolet assay and the microbiological assay, as shown in Table IV. Average results agree within the normal variation of the microbiological assay.

Table IV. Comparison of Ultraviolet Assay and Microbiological Assay on Process Samples

Sample	Assay, γ per Ml.	
	Ultraviolet	Microbiological[a]
Filtered beer	280	290
Filtered beer	330	360
Amyl acetate	2,210	2,270
Aqueous extract	18,100	17,600
Aqueous extract	15,900	16,500
Crude crystals	890	880
Crude crystals	900	900

[a] *Micrococcus pyogenes* var. *aureus* turbidimetric assay.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Ernest Barr, Dorothy Connelly, and Barbara Stalnaker for their technical assistance in preparing this manuscript, and to Harold Tracy for the microbiological assays.

LITERATURE CITED

(1) Barker, S. B., and Summerson, W. H., *J. Biol. Chem.*, **138**, 535 (1941).
(2) Bricker, C. E., and Johnson, H. R., IND. ENG. CHEM., ANAL. ED., **17**, 400 (1945).
(3) Cohen, S. S., *J. Biol. Chem.*, **156**, 691 (1944).
(4) Dische, Z., *Mikrochemie.* **8**, 4 (1930).
(5) Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, **73**, 627 (1927).
(6) Ford, J. H., Prescott, G. C., Hinman, J. W., and Caron, E. L., ANAL. CHEM., **25**, 1195 (1953).
(7) McGuire, J. M., Bunch, R. L., Anderson, R. C., Boaz, H. E., Flynn, E. H., Powell, H. M., and Smith, J. W., *Antibiotics & Chemotherapy*, **2**, 281 (1952).
(8) Perlman, D., *Science*, **118**, 628 (1953).
(9) Pesez, M., *Ann. pharm. franç.*, **10**, 104 (1952).
(10) Pettinga, C. W., Stark, W. M., and Van Abeele, F. R., *J. Am. Chem. Soc.*, **76**, 569 (1954).
(11) Rosenthaler, L., *Chem. Ztg.*, 830 (1912).
(12) Trapp, L., *J. pharm. chim.*, **19**, 295 (1919).

RECEIVED for review June 12, 1954. Accepted December 18, 1954.

Use of a 50-Cm. Heated Gas Cell in Ultraviolet Spectrophotometry

W. F. HAMNER, NINA HADDEN, and W. M. PADGETT II

Monsanto Chemical Co., Texas City, Tex.

This investigation was undertaken to explore the use of a 50-cm. heated gas cell for the detection of trace components in gas streams and the observation of vapor phase spectra of weakly absorbing or low vapor pressure materials. Methods for the analysis of naphthalene in gas streams and benzene in hydrocarbon solvents illustrate the application of the cell to analytical problems. Vapor spectra of naphthalene, phenanthrene, and anthracene were obtained at elevated temperatures and compared with the corresponding solution spectra. The extra path length and higher temperatures provided by the cell increase the versatility of the ultraviolet spectrophotometer.

THE cell compartment of the Cary spectrophotometer is designed to hold cells up to 10 cm. in length. Often a longer path is desired in order to detect trace components in gas samples or to observe the vapor phase spectra of weakly absorbing, or low vapor pressure materials. For most of the problems encountered, a 50-cm. cell designed to operate at elevated temperatures will supply the extra path length required and will allow the vapor pressure of liquids or solids to be increased when necessary to a point where the vapor phase spectra can be obtained. Such a cell has been designed by the Applied Physics Corp. for use with the Cary recording ultraviolet spectrophotometer and has proved itself a versatile analytical aid.

THE CELL

Figure 1 shows the cell in place on the instrument. The cell body and windows are made of fused quartz. Samples are entered through quartz tubulations which extend through the front of the cell housing about 5 cm., and present 18/9 male spherical joints for connection to a suitable handling system.

The optical arrangement for the cell is diagrammed in Figure 2. An image of the monochromator aperture stop is normally formed in the middle of the absorption cell compartment. The cell assembly is placed in the compartment so that the image falls approximately on the lower cell window, which is convex with radius of curvature equal to the cell length. Normally the radiation in the cell compartment is collimated with respect to the slit—that is, the slit image is at infinity. The first toroidal mirror converges the radiation in the cell to form an image of the slit on the upper mirror. The center of curvature of this mirror is approximately at the cell entrance window, so that it forms a second image of the aperture stop, again at the window. The second toroidal mirror has the same power as the first; consequently, the beam emerging from the cell compartment has the same properties whether the cell is present or not, and no refocusing is required when the cell is inserted or removed from the beam. The system is also independent of the refractive index of the substance within the cell (*1*).

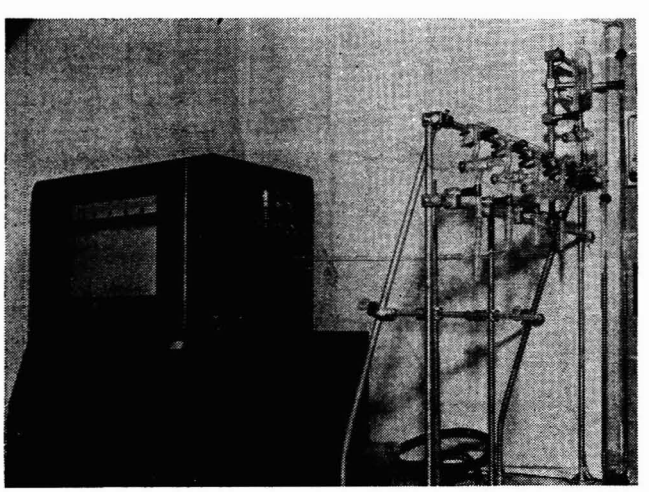


Figure 1. Cary ultraviolet spectrometer, showing 50-cm. cell and gas-handling system

The cell is surrounded by a close-fitting metal tube. Heating is accomplished by tape heaters lying along this tube and held in place with metal cover plates. The allowable range of temperature is 25° to 200° C. Copper tubing for cooling water provides protection for the housing at elevated temperatures.

METHODS OF ANALYSIS

The cell may be evacuated through the upper side arm, which is connected to a high-vacuum gas sampling system. Samples may be entered either from a sample reservoir attached to the lower side arm or through the gas-handling system, in which case the lower side arm is sealed with a glass cap. Entering the sample through the gas-handling system affords a rapid means of gas analysis by which the pressure may be varied and samples pumped in and out without handling the cell. Synthetics may be prepared directly in the cell or vapors passed through for continuous analysis when convenient.

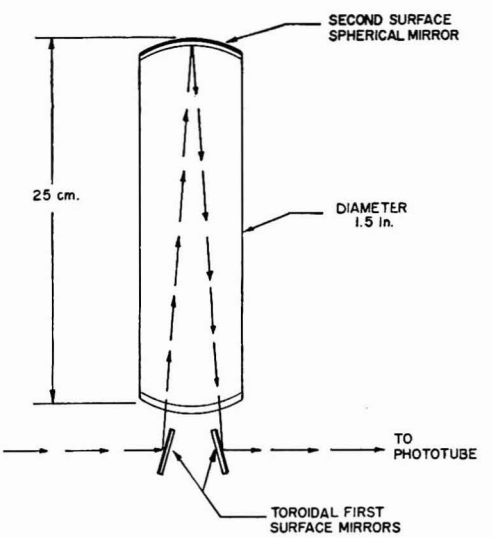


Figure 2. Optical system of 50-cm. cell

Calibration from Vapor Pressure Data. Several sample holders for liquids and solids are illustrated in Figure 3. Container I is a simple freeze-out tip. The sample is placed in the tube, which is fitted to the lower tubulation, and surrounded by liquid nitrogen while the cell is evacuated. The concentration of sample vapor in the cell is, of course, a function of the vapor pressure of the compound and, if the cell temperature is kept above that of the sample reservoir, will be controlled by the reservoir temperature. The reservoir and side arms are heated to the desired temperature with electrical heating tape. Points below room temperature may be obtained by immersing the reservoir in a suitable constant temperature cold bath.

A closer control over temperature is permitted by circulating a liquid of constant temperature through a condenser surrounding the sample tube, as in Figure 3, II. Liquid nitrogen is allowed to enter the condenser while the cell is being evacuated.

A more elaborate apparatus (Figure 3, III), uses a Cottrell pump to control the temperature closely by refluxing a liquid of known boiling point over the sample tube. A coil heater inserted at the bottom of the apparatus provides the necessary heat.

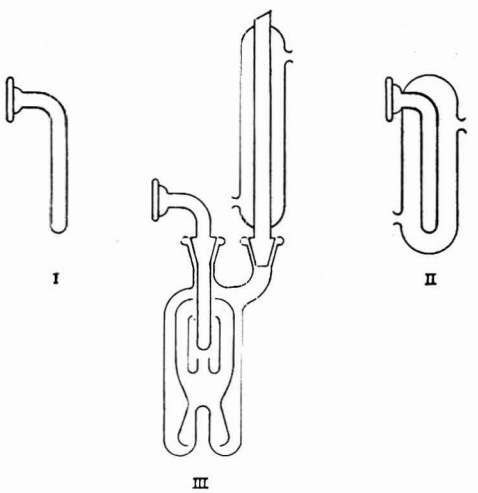


Figure 3. Sample reservoirs for use with 50-cm. cell

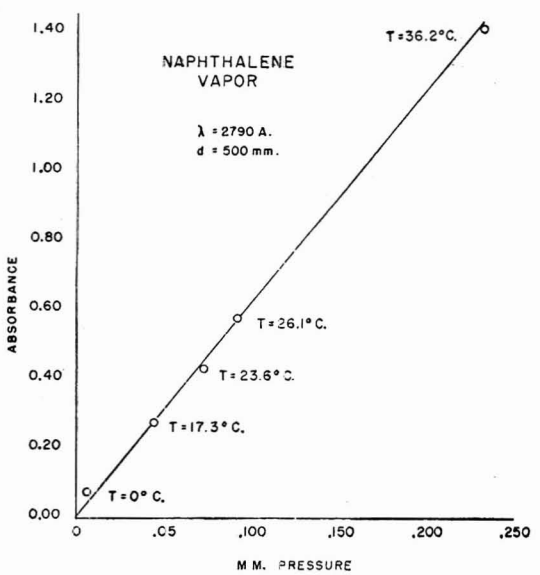


Figure 4. Calibration curve for naphthalene in vapor phase

With vapor pressure–temperature data available, this temperature control of the sample concentration in the cell affords a rapid means of calibration for strong ultraviolet-absorbing materials without the necessity of measuring extremely low pressures. Figure 4 shows a calibration for naphthalene vapor obtained by this method. The portion of the curve from 0.006 to 0.08 mm. of mercury was obtained by measuring the absorbance at 2790 A. with the naphthalene reservoir at the ice point, 17.3° C., and just below room temperature, then plotting absorbance versus the naphthalene vapor pressure in millimeters of mercury. Two points above room temperature were taken. Apparatus II in

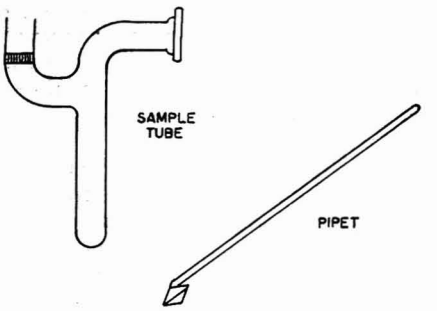


Figure 5. Apparatus for capillary pipet method

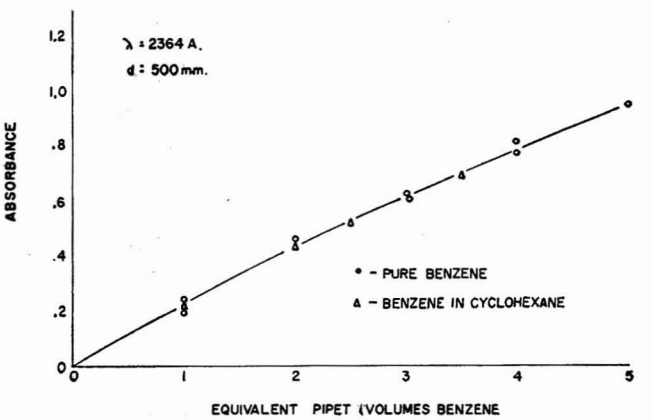


Figure 6. Calibration curve for benzene vapor using capillary pipet method

Figure 3 was used with water from a controlled bath for the 26.1° C. point, and the Cottrell pump with *n*-pentane for the 36.2° C. point. Equilibrium at these temperatures was assumed when a constant absorbance was observed.

Calibration by Capillary Pipet Method. The use of a micropipet for entering liquid samples into an evacuated cell through a sintered-glass disk in order to obtain vapor samples is well known. Figure 5 shows a simple apparatus for using this technique with the 50-cm. cell. The sample is introduced into the evacuated cell through a sintered-glass frit covered by a mercury pool. To accomplish this, and to obtain a constant small vol-

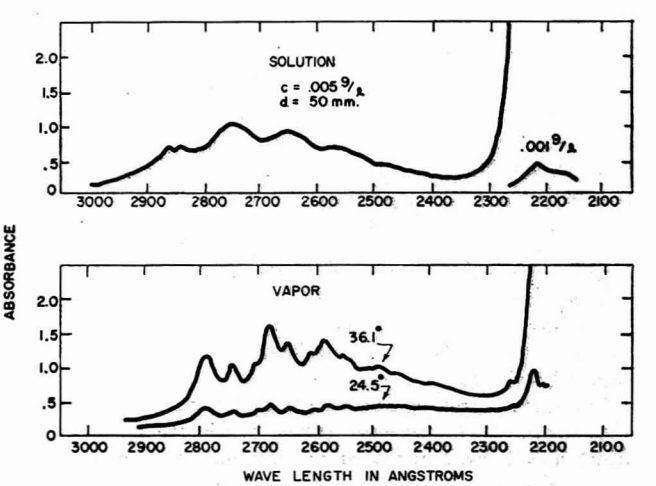


Figure 7. Ultraviolet spectra of naphthalene

ume, a capillary pipet with a ground tip is used. The pipet is filled with sample and lowered through the mercury so as to make close contact with the frit. In this way, the sample is drawn through the frit into the evacuated reservoir, where it is vaporized, and if a second pipetful is to be introduced, frozen out.

Figure 6 shows the curve obtained by introducing from 1 to 5 volumes of pure benzene and from 2 to 7 volumes of a 50-50 benzene-cyclohexane solution in this way and recording absorbance values for the benzene peak at 2365 A. The circles represent points obtained from pure benzene, and the triangles those obtained from equivalent amounts of benzene in the 50% cyclohexane solution. Results of analysis on 30 and 80% mixtures were in error by less than 1% absolute.

This technique has proved valuable in obtaining vapor spectra and in analyzing for components present in solution with hydrocarbon solvents.

VAPOR SPECTRA

The ultraviolet spectrum of a compound in the vapor phase often differs markedly from that of the same compound as a liquid. In general, the bands of the vapor spectrum are better resolved and are shifted toward lower wave lengths.

Figure 7 shows the vapor spectrum of naphthalene obtained by use of the 50-cm. cell. The spectrum of an ethyl alcohol solution is presented for comparison. A shift in absorption between vapor and solution is evident and was also observed for phenanthrene and anthracene, the vapor spectra of which were obtained at temperatures of 100° and 125° C., respectively. This shift becomes greater in the order naphthalene, phenanthrene, anthracene, and in all three cases is larger than the 15-A. shift observed for benzene and monosubstituted benzenes.

The magnitude of the vapor-solution shift observed for a compound is useful in making positive qualitative identification of this compound in a mixture.

LITERATURE CITED

(1) Applied Physics Corp., "Description and Installation Instructions for 50-Cm. Absorption Cell for Use with Cary Recording Spectrophotometer."

RECEIVED for review October 18, 1954. Accepted December 20, 1954. Presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1954.

Spectrophotometric Determination of Gluconic Acid and Its Salts

LESLIE L. ALT

The Diversey Corp., Chicago, Ill.

Gluconic acid and its salts are rapidly acquiring a significant industrial role. These compounds are used under different conditions and may be received in solid or solution form, in an acidic, neutral, or highly alkaline state. One of the most important applications of gluconic acid is in alkaline medium. A procedure which would not require the separation of gluconate from sodium hydroxide seemed to be necessary. Because color is developed in a copper-gluconate complex in alkaline medium, the spectrophotometric method was investigated by measuring the absorbance of this complex. Therefore, a simple and reasonably accurate method was developed which is well suited for analysis of alkaline gluconate samples.

SEVERAL methods were reported in the literature for the determination of gluconic acid and its salts. The polarimetric methods (*1, 3, 6, 11, 12, 14, 23, 24*) utilize the optical rotation of gluconic acid or its complexes with uranium salts, molybdates, or tungstates. Other articles discuss the possibility of determining gluconic acid through oxidation methods (*5, 13, 17, 20*). Removal of the cations by ion exchange, followed by titration with standard base, has been the subject of investigations (*2, 18, 22*). Others have attempted to determine gluconic acid by a gravimetric approach, using phenylhydrazine as the precipitating agent (*7*). There is even an enzymological micromethod (*4*) and a recently developed complexometric titration (*16*). Nonaqueous titrimetric methods (*15, 19, 21*) seem to offer the most convenient approach to the problem. It is beyond the scope of this present work to evaluate the different methods, especially because no extensive comparative work has ever been done in these laboratories.

The rapidly growing number of applications of gluconic acid and its salts (*8–10*) necessitated the development of a method, which may be used even when the gluconate is in highly alkaline solutions. In the author's investigation of complexes of gluconic acid, it was observed that copper forms a relatively stable complex. The absorption spectrum of this complex shows a well-defined absorbance maximum in the visible range. Preliminary examination has also shown that Beer's law is followed within certain concentration limits. Therefore, work was started to develop a practical method.

PROCEDURE

Materials and Apparatus. All the chemicals with the exception of δ-gluconolactone were of reagent grade. The selection of a properly pure salt of gluconic acid presented a problem. It was found, however, that the δ-lactone of gluconic acid (obtained from The Matheson Co., Inc.) may be purified effectively by recrystallizing it from ethylene glycol monomethyl ether. The purity of the recrystallized lactone could not be established by taking its melting point, but had to be determined by a titrimetric method. The weighed sample of the lactone was treated with a known excess of sodium hydroxide solution, followed by titration with a standard acid. This technique was necessary to prevent the introduction of unnecessary errors due to the slow rate of hydrolysis of the lactone in acidic and neutral regions.

Solutions of the lactone as well as those of sodium gluconate are not stable over a long period of time. Preservative had to be added to prevent bacterial action. Ten to 30 mg. of mercuric oxide per liter give satisfactory protection without appreciable interference.

A Beckman DU spectrophotometer was used for the transmittance measurements, using Vycor cells with a 1-cm. path length.

Method I. A sample containing 0.05 to 0.5 millimole of gluconate ion is treated with 15 ml. of 0.1*M* copper sulfate solution. After addition of 10 ml. of 2.5*M* sodium hydroxide, the solution is boiled for 5 minutes, cooled to room temperature, and diluted to 50 ml. Part of this solution is centrifuged at about 2000 r.p.m. for 15 to 20 minutes in stoppered centrifuge tubes. Finally transmittance is determined at 660 mμ, using 0.5*M* sodium hydroxide solution as the blank. Beer's law is obeyed in the 10^{-3} to $10^{-2}M$ concentration range.

Method II. The same size sample (0.05 to 0.5 millimole) is treated with 18 ml. of 1.25*M* sodium hydroxide. Copper sulfate solution (0.1*M*) is added slowly from a 50-ml. buret with adequate stirring of the sample. Stirring is necessary to redissolve the precipitate which is formed during the addition of the copper sulfate solution. As soon as the chelating capacity of the gluconate is exhausted, addition of copper sulfate results in the

formation of a permanent precipitate. This technique will prevent the formation of an undesirable large excess of cupric hydroxide.

The solution is boiled for 5 minutes, cooled to room temperature, and filtered through an ultrafine sintered-glass filter (nominal maximum pore diameter 0.9 to 1.4 microns, obtained from E. H. Sargent & Co., Chicago, Ill.). The filter is washed with 2 ml. of 1.25*M* sodium hydroxide and the filtrate is diluted to 50 ml. This is followed by the transmittance determination at 660 mμ using 0.5*M* sodium hydroxide as the blank.

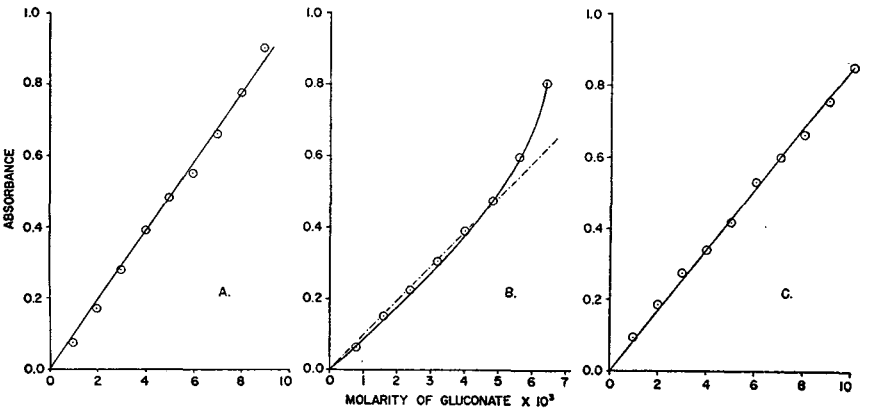


Figure 1. Copper-gluconate complex in 2% sodium hydroxide medium

A. Excess copper hydroxide is removed by centrifuging
B. ——— Filtered sample
— · — · Centrifuged sample
C. Only slight excess of copper sulfate is added. Copper hydroxide is removed by filtration

DISCUSSION OF METHODS

Method I. This method was developed to meet the need of a simple and rapid determination of gluconic acid and its salts.

It has been in use for 2 years and has proved to be a satisfactory control method. Nevertheless, several shortcomings of this method required the development of a more accurate and sensitive method.

First of all, a constant amount of copper sulfate is added to the sample. As the excess cupric ions are precipitated by the alkaline medium a large amount of cupric hydroxide is formed when the gluconate ion concentration is low. The adsorption of the complex by the hydrated hydroxides tends to give low results, especially at lower gluconate ion concentrations.

The removal of the suspended precipitate by centrifuging prior to spectrophotometric measurements is incomplete and erratic. Decrease in transmittance because of the turbidity present gives higher results. Figure 1*A* indicates that Beer's law is followed, as the errors introduced by adsorption and turbidity seem to cancel out each other. Nevertheless, precision is low (±10%).

In the author's experimental work centrifuging was replaced by filtration, a technique which yields brilliant blue solutions. Figure 1*B* shows that the deviation from Beer's law was the result of adsorption losses. Although Beer's law is no longer followed, precision has increased significantly (±0.8 to 1%). The next obvious step was to eliminate the adsorption effect which had led to the development of Method II.

Method II. Reduction of the adsorption of the complex as well as that of turbidity not only increased the precision from ±10% to ±1.5%, but also restored the applicability of Beer's law, as shown in Figure 1*C*.

The concentration of the two reagent solutions used in both methods is not critical. Deviations of ±5% in the concentration of the sodium hydroxide solutions did not seem to affect the results appreciably.

Copper sulfate was not added while preparing the blank solution for no appreciable amount of cuprite ions is formed at this sodium hydroxide concentration.

Heating the solution increases the selectivity of the method, as certain complexes formed by other compounds are thermally unstable. Separation by filtration of the cupric hydroxide is also facilitated by heating, which tends to coagulate colloidal solutions.

Interferences. One of the most serious interferences is caused by the presence of reducing sugars. Their action is twofold. First, on heating or on longer standing they reduce the already formed copper-gluconate complex. Second, the products of this oxidation may form thermally stable copper complexes. It is hardly possible to predict which reaction will be predominant and whether the results will be too low or too high. The mechanism of this two-step reaction appears to be complex; consequently, no predictions can be made without a thorough study of the problem. Therefore, reducing sugars must be removed when present in significant amounts.

Compounds which form stable complexes under the conditions of the method would equally interfere. Aldonic acids, aric acids, polyols, ethylenediaminetetraacetic acid, and similar compounds may be mentioned as examples. In other words the method is not specific for gluconic acid.

Ammonium salts and cyanides will interfere, but their removal is relatively simple. Acetic acid may be used to acidify the sample, followed by boiling, which will remove the hydrogen cyanide. The removal of ammonium salts is much simpler and may be achieved by boiling the sample before the addition of copper sulfate. It is important to cool the sample to room temperature after the aforementioned ions are removed.

The presence of phosphate ion is well tolerated. A solution containing 5×10^{-3} mole of gluconate ion per liter showed a 5.9% decrease in absorbance when the phosphate ion concentration was raised from 0 to $4.5 \times 10^{-3}M$.

It is not necessary to separate the alkali metal hydroxides from the gluconate, provided correction is made in the amount of sodium hydroxide added during the preparation of the sample for spectrophotometric measurement.

The presence of significant amounts of heavy metal ions in general is undesirable, as many of these form stable complexes with gluconic acid.

Other Applications. Work is well under way in these laboratories to adapt the principle of the present method for the determination of copper. Because no copper hydroxide is present in the solution, adsorption and turbidity will not influence the absorbance measurements, and a higher precision than that of the gluconate method is expected.

CONCLUSIONS

Gluconic acid and its salts may be determined spectrophotometrically by using the absorbance of the copper-gluconate complex. This method is not specific for gluconic acid, as the absorbance is due to the central metal atom. Therefore other complexing agents which form similar copper complexes will cause serious interference. Other limiting factors are the adsorption of the complex by the cupric hydroxide precipitate as well as the turbidity due to incomplete removal of the latter.

In spite of its limitations the method may find practical applications especially when gluconate has to be determined in highly alkaline mixtures.

LITERATURE CITED

(1) Bennet-Clark, T. A., *Biochem. J. (London)*, **28**, 45–7 (1934).
(2) Bjorling, C. O., *Farm. Revy*, **48**, 281–5 (1949).
(3) Buogo, G., and Radogna, L., *Boll. chim. farm.*, **86**, 20–3 (1947).
(4) Cohen, S. S., and Raff, R., *J. Biol. Chem.*, **188**, 501 (1951).
(5) Courtois, J., and Wickstrom, A., *Ann. pharm. franç.*, **7**, 288 (1949).
(6) DeCarli, F., *Ann chim. appl.*, **21**, 465–71 (1931).
(7) Diemair, W., Bleyer, B., and Schneider, L., *Z. Untersuch. Lebensm.*, **69**, 212–20 (1935).
(8) Dvorkovitz, V., and Hawley, T. G. (to The Diversey Corp.), U. S. Patent **2,584,017** (Jan. 29, 1952).
(9) *Ibid.*, **2,615,846** (Oct. 28, 1952).
(10) Dvorkovitz, V., and Swihart, J. R. (to The Diversey Corp.), *Ibid.*, **2,650,875** (Sept. 1, 1953).
(11) Fisher, H. J., *J. Assoc. Offic. Agr. Chem.*, **16**, 379–83 (1933).
(12) Fisher, H. J., and Bailey, E. M., *Ibid.*, **15**, 461–6 (1932).
(13) Fleury, P., Courtois, J., and Wickstrom, A., *Ann. pharm. franç.*, **6**, 338–47 (1948).
(14) Fungairino, L. V., *Monit. farm. y terap. (Madrid)*, **55**, 551–3 (1949).
(15) Izmailov, N. A., and Bezuglyi, V. B., *Med. Prom. S.S.S.R.*, **1949**, No. 2, 23–5.
(16) Karabinos, J. V., Ballun, A. T., and McBeth, R. L., ANAL. CHEM., **25**, 1563 (1953).
(17) Lucas, V., *Bol. assoc. brasil. farm.*, **17**, 10–16 (1936).
(18) Marconi, M., *Chimica (Milan)*, **6**, 384 (1951).
(19) Markunas, P. C., and Riddick, J. A., ANAL. CHEM., **23**, 337 (1951).
(20) Sas, F. E. R., Hernandez-Guttierez, F., and Cofino, M. C., *Anales real soc. españ. fis. y quim. (Madrid)*, **45B**, 1277–313 (1949).
(21) Spengler, H., and Kaelin, A., *Hundert Jahre Schweiz. Apoth. Ver. (Centenaire soc. suisse pharm.)*, **1843–1943**, 542–64 (1943).
(22) Vaisman, G. A., and Yampol'Skaya, M. M., *Zavodskaya Lab.*, **16**, 621–2 (1950).
(23) Vintilescu, I., Ionescu, C. N., and Stanciu, N., *Bul. Soc. Stiinte Farm. Romania*, **3**, 334–42 (1938).
(24) Vintilescu, I., Ionescu, C. N., and Stanciu, N., *J. pharm. chim.*, **28**, 283–93 (1938).

RECEIVED for review September 29, 1954. Accepted December 18, 1954. Presented before the Division of Carbohydrate Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York.

Spectrographic Analysis of Briquetted Unashed Plant Material

J. H. MUNTZ and S. W. MELSTED

Department of Agronomy, University of Illinois, Urbana, Ill.

A rapid spectrographic method suitable for routine analysis of a large number of plant samples has been developed. The sample is prepared by mixing finely ground plant material with lithium carbonate and graphite and briquetting the mixture. Excitation is by the high voltage spark. Eight elements can be determined in a single exposure with an accuracy within about ±20%.

IN MANY instances the time consumed in preparing the plant sample for spectrographic analysis nullifies the greater speed usually associated with this type of analysis. This paper discusses a technique for preparing plant material for spectrographic analysis, using an unashed briquetted sample. This technique is rapid and adaptable to routine work.

Spectrographic analysis is used frequently for determining the mineral composition of plant material. Mathis (*2*), Mitchell (*4*), Vanselow and Liebig (*7*), and Sayre (*6*) have published methods for the spectrographic analysis of plants. Techniques usually follow one of three general types: analysis of plant ash as such, analysis of a solution of the ash placed on an electrode, and analysis of a chemically concentrated aliquot of the sample. The use of a compressed pellet or briquetted sample is not common in plant analysis, although the briquet has proved successful for metallic samples and for some nonmetallic samples such as cement (*1*) and cracking catalyst materials (*5*). Wilson (*8*) and Milbourn (*3*) make reference to the use of compressed pellets, Wilson using "sulfated ash" and Milbourn using unashed plant material.

Preliminary work with unashed briquetted plant samples for the determination of boron indicated the possibility of using this method of sample preparation for a rapid spectrographic determination of most of the mineral elements of interest in plant material. Eight elements—phosphorus, magnesium, calcium, potassium, manganese, boron, copper, and zinc—were determined by the method described. Four others—sodium, silicon, iron, and aluminum—were detected and could have been determined if adequate standards had been available. Two others of interest—cobalt and molybdenum—are present in most plant materials in amounts below the sensitivity of the method.

Table I. Spectral Lines Used for Analysis

Element	Spectral Line	Sector Step
Boron	2497.73	1
Phosphorus	2553.28	1
Manganese	2576.10	3
Lithium	2741.31	5
Magnesium	2782.97	2
Calcium	3158.87	6
Copper	3273.96	2[a]
Zinc	3345.02	2[a]
Potassium	3446.72	2[a]

[a] Background correction made.

APPARATUS

Hilger large Littrow quartz spectrograph.
N.S.L. Spec Power source unit, alternating current spark section (National Spectrographic Laboratories).
A.R.L. briquetting press (Applied Research Laboratories)
A.R.L. developing equipment.
Jarrell-Ash microphotometer.

PROCEDURE

Because of the impracticability of preparing synthetic standards that would approximate unashed plant material, all standards were plant samples that had been chemically analyzed. Twelve to 20 samples were used as standards for the preparation of each working curve. Whole plant samples of immature wheat, rye, red clover, alsike clover, alfalfa, and tall oat grass were used for phosphorus, magnesium, calcium, and potassium. Samples of immature alfalfa, corn, and soybean leaves served as standards for boron, copper, and zinc, respectively. Immature wheat, rye, and tall oat grass were used for manganese.

The samples were prepared by mixing 150 mg. of finely ground plant material with 75 mg. of lithium carbonate by grinding in an agate mortar. Four hundred and fifty milligrams of SP-1 graphite (National Carbon Co.) were added and mixed in. The mixture was then briquetted in a 0.5-inch briquetting press at a pressure of 100,000 pounds per square inch. Lithium carbonate serves as both a buffer and an internal standard. Graphite was used to render the briquet electrically conductive. A high voltage spark was the source of excitation, with the following conditions employed:

Capacitance, 0.01 microfarad.
Inductance, 300 microhenries.
Primary voltage, 200 volts.
R.F. amperage, 7.
Breaks, 3.
Auxiliary gap, 5 mm.
Air pressure, 1.9 pounds.

Exposure
Total time, 100 seconds.
Prespark, 10 seconds.
Electrodes
Lower, sample briquet.
Upper, 1/16-inch rod, 120° cone tip.
Gap, 2 mm.

Table II. Analysis of Mixtures of Samples That Vary in Composition and Physical Characteristics[a]

	1:1 Ratio			1:3 Ratio			3:1 Ratio		
	Average present[b]	Amount found[c]	Difference	Average present[b]	Amount found[c]	Difference	Average present[b]	Amount found[c]	Difference
				Corn Grain–Alfalfa					
	%	%	%	%	%	%	%	%	%
P	0.22	0.24	8	0.17	0.18	6	0.23	0.26	13
Mg	0.33	0.30	9	0.42	0.45	7	0.23	0.25	9
Ca	0.78	0.70	10	1.14	1.21	6	0.42	0.35	17
K	0.61	0.72	18	0.65	0.78	20	0.57	0.74	30
	P.p.m.	P.p.m.		P.p.m.	P.p.m.		P.p.m.	P.p.m.	
B	14	14	0	19	19	0	12	12	0
Mn	34	28	18	47	37	21	22	17	23
Cu	8.3	8.2	1	10.1	10.0	1	6.4	6.4	0
Zn	24	27	13	25	26	4	24	26	8
				Rye–Apple Leaves					
	%	%		%	%		%	%	
P	0.33	0.36	9	0.24	0.24	0	0.41	0.42	3
Mg	0.35	0.35	0	0.38	0.32	16	0.31	0.29	7
Ca	1.47	1.70	16	1.99	2.35	18	0.96	1.07	11
K	1.86	1.82	2	1.37	1.45	6	2.36	2.20	7
	P.p.m.	P.p.m.		P.p.m.	P.p.m.		P.p.m.	P.p.m.	
B	40	41	3	31	32	3	48	48	0
Mn	176	175	1	183	180	2	169	183	8
Cu	10.6	10.8	2	9.8	11.0	12	11.5	11.5	0
Zn	45	42	7	48	49	2	42	42	0
				Soybean Leaves–Wheat					
	%	%		%	%		%	%	
P	0.16	0.18	11	0.15	0.19	27	0.17	0.19	12
Mg	0.34	0.28	18	0.21	0.18	14	0.47	0.38	19
Ca	1.05	0.80	24	0.62	0.51	18	1.49	1.15	23
K	1.23	0.97	21	1.34	1.05	22	1.11	0.95	14
	P.p.m.	P.p.m.		P.p.m.	P.p.m.		P.p.m.	P.p.m.	
B	32	34	6	28	31	11	37	37	0
Mn	630	680	8	370	430	16	890	900	1
Cu	8.6	9.4	9	8.1	7.4	9	9.1	9.6	6
Zn	89	96	8	54	58	7	125	117	6

[a] Immature whole plant samples unless otherwise specified.
[b] Calculated average based on the spectrographic analysis of the individual components.
[c] Average of duplicate determinations.

Plant samples vary greatly in physical characteristics and in mineral and organic composition, even within a single species. Such differences may cause variations in excitation conditions and lead to erroneous results. Both the buffer and the graphite would tend to minimize the possibility of such errors. In order to test the method in this regard, trials were made with mixtures of samples that differed in composition and physical characteristics. Mixtures of corn grain–alfalfa, rye–apple leaves, and soybean leaves–wheat in ratios of 1 to 1, 1 to 3, and 3 to 1 were analyzed and compared with the calculated average of the spectrographic analysis for individual components. (Immature whole plant samples were used unless otherwise specified.) Table II shows that with but few exceptions the analysis of the mixture agrees with the calculated composition for the mixture, within the precision limits of the method. The data in Table II also indicate that the briquetting technique is overcoming such differences in plant composition and physical characteristics that might cause variations in excitation conditions.

Exposure was made on a Koda k S.A. No. 1 plate, 4 × 10 inches, using a seven-step filter to sector the emitted light. The plate was developed and the line densities were then determined for the spectral lines given in Table I. These lines were chosen so all eight elements could be determined with a single exposure. Line density was converted to relative intensity by means of an emulsion calibration curve. Intensity ratios of analysis lines to lithium lines were used in the usual manner for preparing working curves and determining concentrations. Standard samples were used to check for possible shifts in the working curves.

DISCUSSION

Several different ratios of sample to buffer to graphite were tried before the ratio of 2 to 1 to 6 was selected. This amount of buffer was about the maximum permissible without excessive suppression of the analysis lines. The amount of graphite was found to be about the minimum for forming briquets with good sparking characteristics and about the maximum that could be used without unduly decreasing sensitivity. Lines of the elements phosphorus, zinc, and potassium were weak and increasing the amount of either buffer or graphite to any extent would have prevented their use under the sparking conditions employed.

The briquetting pressure is important, because samples vary in compressibility. Unless fairly high pressure is used, the briquets will not have uniform sparking surfaces.

An indication of the precision and accuracy of the method is presented in Table III. Comparisons are made between spectrographic determinations and chemical data obtained from another laboratory. Standard deviations between the methods and between duplicate spectrographic determinations for the same samples are indicated. Usually duplicates will agree within 20% or less and reruns should be made if they do not. From the standard deviations between duplicates it can be seen that the method is not as precise for the determination of phosphorus, zinc, and potassium as for the other elements. This is probably due to the weak spectral lines of these elements at the concentration levels

Table III. Comparison of Spectrographic and Chemical Analysis

	Chem.	Spec.	Diff.	Chem.	Spec.	Diff.	Chem.	Spec.	Diff.	Chem.	Spec.	Diff.
	Boron			Phosphorus, %			Manganese			Magnesium, %		
Sample	P.p.m.	P.p.m.	%				P.p.m.	P.p.m.	%			
1	26	19	27	0.17	0.19	12	47	52	11	0.20	0.26	30
2	26	19	27	0.14	0.15	7	45	43	5	0.20	0.24	20
3	26	20	23	0.14	0.16	14	66	55	17	0.18	0.21	17
4	30	22	27	0.16	0.23	44	53	52	2	0.31	0.36	16
5	26	19	27	0.15	0.17	13	45	46	2	0.36	0.40	11
6	26	19	27	0.14	0.16	14	72	70	3	0.18	0.24	33
7	34	25	27	0.16	0.17	6	40	48	20	0.26	0.30	15
		5.35[a]			0.022[a]			3.96[a]			0.032[a]	
		1.05[b]			0.023[b]			2.50[b]			0.017[b]	
	Calcium, %			Copper			Zinc			Potassium, %		
				P.p.m.	P.p.m.	%	P.p.m.	P.p.m.	%			
1	1.11	1.05	5	15.3	15	2	22.0	27	23	1.61	1.68	5
2	1.18	1.24	5	17.0	20	18	21.5	30	40	1.42	1.16	18
3	1.37	1.24	10	17.8	19	7	30.0	34	13	1.74	1.32	24
4	1.77	1.92	9	15.8	19	20	21.5	36	68	1.13	1.31	16
5	1.03	1.10	7	16.0	17	6	20.0	26	30	1.80	1.80	0
6	1.28	1.52	19	17.3	18	4	19.0	33	74	1.29	1.18	9
7	1.36	1.48	9	16.5	19	15	21.0	35	67	0.77	0.72	7
		0.086[a]			1.40[a]			7.3[a]			0.140[a]	
		0.057[b]			1.52[b]			7.1[b]			0.184[b]	

[a] Standard deviation between methods.
[b] Standard deviation between spectrographic duplicates.

found in these samples. Reruns were more often necessary for these elements. An over-all accuracy within about ±20% was obtained for all elements, with the exception of zinc. Greater accuracy should be obtainable for zinc at higher concentrations.

The spectrographic determinations for phosphorus, magnesium, copper, and zinc were consistently higher than the chemical data obtained from another laboratory. This would indicate a need for shifting the working curves, provided the chemical data from the other laboratory are more accurate than the chemical data used as standards in preparing the working curves.

LITERATURE CITED

(1) Helz, A. W., and Scribner, B. F., *J. Research Natl. Bur. Standards*, **38**, 439 (1947).
(2) Mathis, W. T., ANAL. CHEM., **25**, 943 (1953).
(3) Milbourn, M., *J. Soc. Chem. Ind. (London)*, **56**, 205T (1938).
(4) Mitchell, R. L., Commonwealth Bur. Soil Sci., *Tech. Commun.* 44 (1948).
(5) Russell, R. G., ANAL. CHEM., **20**, 296 (1948).
(6) Sayre, J. D., Ohio Agr. Expt. Sta., Mimeo.
(7) Vanselow, A. P., and Liebig, G. F., Calif. Agr. Expt. Sta., Berkeley, Calif., Mimeo. Publ. (1948).
(8) Wilson, S. H., *Roy. Australian Chem. Inst. J. & Proc.*, **9**, 205 (1942).

RECEIVED for review October 5, 1954. Accepted January 17, 1955.

Spectrographic Determination of Lead in Oxygen-Free, High-Conductivity Copper

SAMUEL B. DEAL

Tube Division, Radio Corp. of America, Lancaster, Pa.

This paper describes use of metal standards for the quantitative spectrographic determination of lead in oxygen-free, high-conductivity copper. The method consists of direct arcing of metal samples and standards, photometry of the resulting spectral lines chosen as analytical lines, and the preparation of a working curve for determination of concentration of lead. A procedure is given for the selection and colorimetric establishment of metal standards. The equipment used includes a large Bausch & Lomb Littrow spectrograph, an alternating current power supply, an ARL–Dietert densitometer, and a calculating board. The method is particularly well suited for production control.

THE spectrographic technique provides a rapid method for the quantitative determination of very small amounts of lead in oxygen-free, high-conductivity copper. Although two other common methods of analysis, the colorimetric and the polarographic, are of comparable sensitivity, each of these methods requires preliminary complexing or removal of copper. This separation of copper is time-consuming, and special precautions are required because of the use of cyanide as a complex-forming agent. The spectrographic method eliminates these objectionable features.

Some methods for the spectrographic determination of lead in copper are given in existing literature. None of these methods, however, presents a procedure based on direct arcing of the metal and the use of metal standards.

In 1933, Breckpot (*1*) studied the behavior, intensities, and characteristics of spectral lines produced by concentrations of lead from 0.001 to 1% in copper, and concluded that the most persistent lead line occurred at 2833.1 A. As a result of these studies, Breckpot developed a quantitative method (*3*) based on the use of copper oxide powders, which he prepared from copper solutions because he could not obtain alloys of known composition. This method was used for the analysis of lead in technical and electrolytic copper (*4*). Breckpot also used coprecipitation of lead with hydrous ferric oxide (*2*) prior to evaporation in an electrode as a means for improvement.

Ratsbaum (*7*) employed an intermittent arc for determination of lead in electrolytic copper, using a solution method. In 1935, Park and Lewis (*6*) employed a solution technique which used liquid standards and direct comparison of standard and sample spectrograms. Jaycox and Ruehle (*5*) used a solution sample and rotating sample electrode. By preparing working curves from liquid standards, they were able to obtain fairly good results.

The method described in this paper eliminates the inconvenience of liquid standards, dissolving of the sample, and preliminary chemical separations, and provides an accurate quantitative method.

EXPERIMENTAL INVESTIGATION

A major portion of the copper samples for which quantitative determination of lead was required were in the form of small tubing, a sample shape which is not readily adaptable to the use of a spark source for excitation. In addition, the maximum allowable concentration of lead in the copper samples was near the lower sensitivity limit of the spark spectrum.

A direct current arc was too sensitive for the lead concentration range under investigation, and produced lead spectral lines having a greater density than that desired for the most accurate results. An alternating current arc, therefore, was chosen as the exciting source unit because the persistent lead spectral lines in the spectral region used were of approximately the correct intensity for precise quantitative measurement and showed better reproducibility.

The use of an alternating current power source in the arcing of copper containing lead resulted in nonuniform results on samples of varying sizes possibly because of variation in rate of lead volatilization. By means of the "moving-plate" technique, it was found that a 55-mg. sample of copper containing a small amount of lead lost a large portion of this lead during the first minute of arcing time; practically all of the lead was gone after 3 minutes. When a smaller quantity of copper (10 mg.) was arced and the spectra were recorded on a moving photographic plate, all spectrographically detectable lead was recorded within 20 seconds. The 10-mg. sample also produced a series of lead lines having more appropriate intensity for subsequent photometry. A final sample size of 5 mg. was used because the intensity of the lead line chosen for density measurement was of the best order of magnitude with a sample of this size. The lower, or sample-containing, electrode was cratered to a very shallow depth, approximately 0.8 mm., to minimize the introduction of background on the photographic plate used to record the spectral lines.

PREPARATION OF STANDARDS

A series of copper samples having known lead contents in the range of 0.0005 to 0.005% was required for use as standards in the preparation of a working curve. After a number of copper samples had been recorded spectrographically, the lead lines chosen as the analytical lines were compared visually with the aid of a reading glass having a magnification factor of 4. Three

samples exhibiting lead lines of low, medium, and high intensity, respectively, were selected as standards.

The three copper samples to be used as standards were analyzed by the dithizone (*8*) colorimetric method for lead content, and were found to contain 0.00049, 0.0009, and 0.0019% lead, respectively. A quantity of copper representative of each of the three samples was reserved for use as standards in the preparation of working curves for routine analysis.

CONDITIONS

The equipment used in this spectrographic method and the conditions for analysis were as follows:

Spectrograph, large Bausch & Lomb Littrow.

Densitometer, Applied Research Laboratories–Dietert.

Sample, 5 ± 0.1 mg., weighed on a micro torsion balance having a capacity of 15 mg.

Electrodes, graphite spectrographic electrodes, 3.2 × 32 mm.; upper or counter electrode tapered to 30 degrees; lower or sample electrode cratered to a depth of approximately 0.8 mm.

Electrode gap, approximately 5 mm.

Burning time, 30 seconds.

Power source, alternating current arc; 2.5-kv. secondary voltage, 40- to 50-volt arc voltage; 3.5-ampere arc current.

Slit, 0.010 mm., fixed.

Diaphragm, larger of two diaphragms positioned in front of prism.

Photographic plates, Kodak Spectrum Analysis No. 1.

Developer and developing time, D-19 developer for 3 minutes; 1% acetic acid short-stop for 1 minute; fixer for 3 minutes. Self-agitating type developing tank.

Spectral lines, 2833.1-A. lead line; 2858.7-A. copper line. Fifth spectral region, in the spectral range from 2500 to 3400 A.

A calculating board was used for determining background corrections and intensity ratios.

Table I. Reproducibility of Results

Sample No.	Log (Intensity of Lead)/(Intensity of Copper)	Lead, %
1	6.0	0.0013
	5.8	0.0012
	5.5	0.0012
	6.3	0.0014
	5.5	0.0012
	6.2	0.0014
2	4.4	0.0008
	4.2	0.0008
	4.5	0.0008
	4.7	0.0009
	4.4	0.0008
	4.6	0.0009
3	7.8	0.0020
	7.6	0.0019
	7.8	0.0020
	8.0	0.0021
	8.2	0.0021
	7.9	0.0020

DETERMINATION OF LEAD

Weighed samples of copper were used to minimize variation in rate of lead volatilization. The copper was cut to the proper size with a pair of steel clippers used only for this type of material, and a 5-mg. sample was weighed on the torsion balance.

In this method of analysis, sample loss was minimized by initiating sample excitation with the two electrodes immediately adjacent to each other. When the arc was started between the electrodes, the analytical gap was adjusted rapidly and the copper sample was arced to completion. Before this technique was used, frequent loss of sample was experienced as a result of the sample being ejected from the shallow lower-electrode crater by the sudden burst of electrical energy.

Figure 1 shows a typical working curve obtained by plotting the log intensity ratios of the 2833.1-A. lead line to the 2858.7-A. copper line against the log concentration of lead. The curve approaches a straight-line function having a slope which closely approximates the theoretical 45° slope.

Some results obtained by the quantitative spectrographic analysis of oxygen-free, high-conductivity copper samples are given in Table I. Reproducibility of results is also demonstrated.

A comparison of spectrographic results and those obtained by the dithizone colorimetric method is shown in Table II.

Table III gives a comparison of results obtained by spectrographic analysis at RCA with those obtained by an outside laboratory on the standards described above and on several copper samples. The method used at the outside laboratory is similar to the one described in this paper except that a direct current arc is employed for sample excitation and a 100-mg. sample is used. A special spectrographic design is used to reduce background. Standards were analyzed at the outside laboratory by a carrier technique which is entirely different from the dithizone method used here.

Table II. Comparison of Spectrographic and Colorimetric Results

Lead, %	
Spectrographic method	Colorimetric method
0.0006	0.0005
0.0009	0.0008
0.0012	0.0010
0.0015	0.0014
0.0018	0.0018
0.0021	0.0020

Table III. Comparison of Analyses of Standards and Samples by RCA and Outside Laboratory

Sample No.	Lead, % RCA analysis	Lead, % Outside laboratory analysis
Std. *A*	0.00049	0.00050
Std. *B*	0.0006	0.00063
Std. *C*	0.0019	0.0018
No. 1	0.002	0.0022
No. 2	0.0015	0.00145
No. 3	0.0005 or less	0.00042
No. 4	0.001	0.0013
No. 5	0.00086	0.00088

CONCLUSIONS

The quantitative spectrographic method affords an accurate and relatively rapid method for the determination of small amounts of lead in oxygen-free, high-conductivity copper. Although a weighing operation is involved in the preparation of the samples, the over-all time of analysis is quite short. This method has proved to be reliable during the 3-year period in which it has been in use.

ACKNOWLEDGMENT

The author wishes to acknowledge the assistance of Jeanette E. Cooper of the RCA Tube Division in performing a major

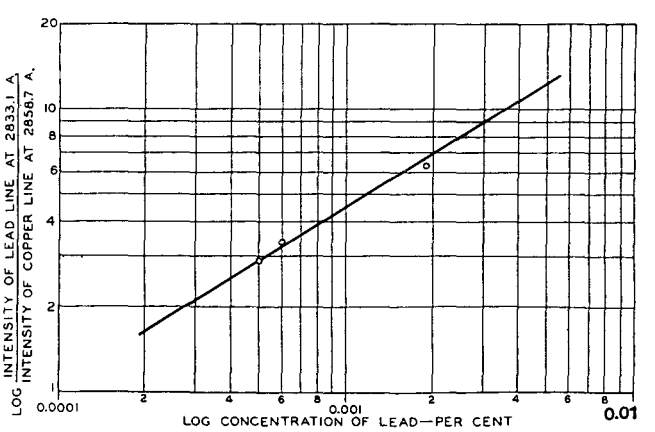


Figure 1. Lead working curve

portion of the spectrographic analyses, and the cooperation of R. G. Ernst and W. E. Publicover of the U. S. Metals Refining Co. in performing comparison analyses.

LITERATURE CITED

(1) Breckpot, R., *Ann. soc. sci. Bruxelles*, **B53**, 219–47 (1933).
(2) *Ibid.*, **55B**, 173–94 (1935).
(3) Breckpot, R., *Chimie & industrie*, Special No., 220–9 (April 1934).
(4) Breckpot, R., *Natuurw. Tijdschr.*, **16**, 139–43 (1934).
(5) Jaycox, E. K., and Ruehle, A. E., IND. ENG. CHEM., ANAL. ED., **12**, 195–6 (1940).
(6) Park, B., and Lewis, E. J., *Ibid.*, **7**, 182–3 (1935).
(7) Ratsbaum, E. A., *Zavodskaya Lab.*, **6**, 191–5 (1937).
(8) Sandell, E. B., "Colorimetric Determination of Traces of Metals," Vol. III, pp. 282–9, Interscience, New York, 1944.

RECEIVED for review October 7, 1954. Accepted December 22, 1954.

Ultraviolet Determination of Combined Methyl Isopropenyl Ketone in Polymers

J. J. PEPE, IRWIN KNIEL, and MICHAEL CZUHA, JR.
Government Laboratories, University of Akron, Akron, Ohio

This work was undertaken in connection with pilot plant studies on methyl isopropenyl ketone polymers. To correlate physical test data of these polymers with their chemical composition, the methyl isopropenyl ketone content was determined by ultraviolet spectroscopy. This method is based upon the low absorptivity of polybutadiene and butadiene-acrylonitrile copolymers at the absorption maximum for methyl isopropenyl ketone polymers in chloroform. The absorption maximum of the polymers containing methyl isopropenyl ketone occurs at a wave length of 290 mμ at a slit width of 0.5 mm. on the Beckman Model DU spectrophotometer, while the absorptivity of polybutadiene appears to be a minimum value at this wave length. The difference between the total absorptivity and absorptivity due to butadiene or butadiene-acrylonitrile copolymers against chloroform as a solvent blank represents absorptivity due to combined methyl isopropenyl ketone. This method is rapid and reproducible to within 0.4% methyl isopropenyl ketone, based upon the average of quadruplicate determinations for each polymer studied. Wherever possible, products not stabilized with antioxidants should be employed for analysis to circumvent interference from phenyl-2-naphthylamine.

A RAPID method providing reproducible results for the determination of combined methyl isopropenyl ketone in butadiene–methyl isopropenyl ketone (BD/MIK) copolymers and butadiene–methyl isopropenyl ketone–acrylonitrile tripolymers is imperative for the study of the chemical and physical properties of these polymers.

A transmittance curve drawn from the observed values of a sample containing 1 gram of methyl isopropenyl ketone polymer per liter of chloroform gave an absorption maximum at a wave length of 290 mμ. This maximum is shifted slightly from the known absorption maximum, at a wave length of 280 mμ for organic compounds containing a carbonyl group (*2*). The absorptivity, a, defined by the equation

$$a = \frac{A}{c'b}$$

where A is the logarithm to the base 10 of the reciprocal of the transmittance, c' is grams of solute per liter, and b is the length of the optical path. The absorptivity for methyl isopropenyl ketone polymer, which obeys Beer's law, was calculated to be 0.570 liter per gram for concentrations of 0.1 to 1.0 gram per liter.

The absorptivity of polybutadiene was determined to be 0.010 and, for butadiene–acrylonitrile (BD/AN) polymers of variable charge ratio, the absorptivity was found to be 0.0097. Thus, the absorptivity for polybutadiene is nearly equal to that for butadiene–acrylonitrile polymer and the value of 0.01 was used for both. Assuming that the contribution of combined methyl isopropenyl ketone (MIK) to the absorbance of methyl isopropenyl ketone homopolymer is the same as that in the butadiene–methyl isopropenyl ketone copolymer and that for the butadiene–acrylonitrile–methyl isopropenyl ketone tripolymer, at constant weights of methyl isopropenyl ketone at a wave length of 290 mμ the following derivation was made; a represents absorptivity:

$$a_{\text{Total}} = a_{\text{MIK}} + a_{\text{BD}}$$

or

$$a_{\text{Total}} = a_{\text{MIK}} + a_{\text{BD/AN}}$$

and

$$a_{\text{MIK}} = a_{\text{Total}} - a_{\text{BD}} \qquad (1)$$

Let x equal the weight fraction of combined methyl isopropenyl ketone and $(1-x)$ equal the weight fraction of combined butadiene or combined butadiene–acrylonitrile in the polymer, then:

$$a_{\text{Total}} = 0.570x + 0.010(1 - x) \qquad (2)$$

or

$$x = 1.785\, a_{\text{Total}} - 0.018$$

and

$$\%\ \text{MIK} = 178.5\, a_{\text{Total}} - 1.8 \qquad (3)$$

where

$$a_{\text{Total}} = \frac{\text{Absorbance (from instrument)}}{\text{concentration (g./liter)}}$$

PROCEDURE

The procedure that was used to explore the spectrophotometric method for determination of combined methyl isopropenyl ketone is described below.

Reagents and Apparatus. Chloroform, C.P. or redistilled, having a transmittance of 91 to 100% based upon distilled water as 100% at 290 mμ.

Methanol, technical grade.

Chloroform, technical grade.

Mixed solvent. The solvent solution is made up by adding 20 parts of methanol to 80 parts of technical grade chloroform.

Ethyl alcohol, 3A or 2B (denatured grade).

High speed blender.

Beckman quartz spectrophotometer, Model DU, and accessories.

Seitz filter apparatus. A brass silver-plated filter provided with filter disks for rapid filtration of 100-ml. quantities of serum type solutions (*1*). Vacuum or pressure may be used to filter.

Prepared filter disks, 60-mm., round filter disks that have been soaked in chloroform.

Nitrogen, compressed gas.

Purification of Polymer. Weigh into a 4-ounce bottle a 0.6-gram sample of raw polymer and add 100 ml. of solvent solution.

Shake the bottle on a mechanical shaker until the sample has been dissolved. Transfer the sample from the 4-ounce bottle to a 125-ml. separatory funnel. Next, add 300 ml. of methanol to the high speed blender. Set the blender to medium speed and slowly add the polymer solution to the methanol in the blender. Remove the glass container from the blender. Scrape the sides of the glass with a stainless steel spatula to remove adhering polymer. Filter the precipitated polymer onto a 9-cm., No. 42 Whatman filter paper in a Büchner funnel. Wash the filtered polymer with two 25-ml. portions of methanol. The washed polymer, which should appear white, is then transferred to the original 4-ounce bottle; 80 ml. of C.P. chloroform are added, and the bottle is shaken until the polymer has dissolved but may contain transparent gel.

Preparation of Sample. The sample is transferred to a Seitz filter apparatus which has been fitted with a prepared filter disk and operates under a nitrogen pressure of 60 pounds per square inch applied from a cylinder. The filtrate is collected in a 100-ml. volumetric flask and diluted to the mark with C.P. chloroform. Next, two 25-ml. aliquots are taken from the flask and transferred to tared aluminum dishes. The dishes are placed on a hot plate and the chloroform is evaporated at about 50° C. The dry dishes are then reweighed; the increase in weight times 40 is the concentration of polymer in grams per liter. The average of duplicate determinations should not vary by more than 2%. A concentration of 2 to 3 grams per liter gives satisfactory results. Further dilution may be necessary to attain this concentration.

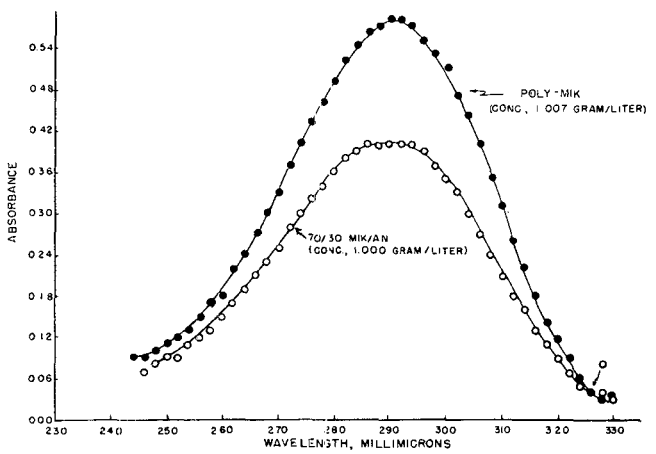


Figure 1. Absorption spectra of poly(methyl isopropenyl ketone) and 70/30 methyl isopropenyl ketone–acrylonitrile copolymer

Slit width, 0.5 mm.

Spectrophotometric Measurement. The slit width of the spectrophotometer is set at 0.5 mm. and the wave length at 290 mμ. The sample and the solvent used for preparation of the sample are placed in quartz cells, 1 cm. square, and the absorbance of the sample is determined, as compared with zero absorbance for a chloroform blank. Since the concentration and absorbance are known, the absorptivity can be calculated and from this value the percentage of combined methyl isopropenyl ketone can be determined by Equation 3.

RESULTS AND DISCUSSION

A rapid and reproducible method for the determination of combined methyl isopropenyl ketone in butadiene–methyl isopropenyl ketone copolymers and in butadiene–acrylonitrile–methyl isopropenyl ketone tripolymers was developed after study of the spectrophotometric behavior of the monomers, copolymers, and tripolymers at various wave lengths, with a slit opening of 0.5 mm., as shown in Figure 1. Methyl isopropenyl ketone polymer in chloroform has an absorption maximum at 290 mμ. The absorptivity curve for butadiene polymer, as shown in Figure 2, shows no inflection at 290 mμ. Table I shows further that the absorptivity of polybutadiene remains practically constant (0.010) at the concentrations employed. These data indicated

Table I. Spectrophotometric Measurement on Polybutadiene[a]

Concentration, Grams/Liter	Absorbance	Absorptivity[b]
2.20	0.023	0.010
2.40	0.025	0.010
2.55	0.026	0.010
2.55	0.026	0.010
2.60	0.027	0.011
2.10	0.021	0.010
2.00	0.020	0.010
3.20	0.030	0.009
	Average	0.010

[a] Polybutadiene was prepared in 8-ounce bottles at 104° F. to a conversion of 58.3%, and purified as described under Procedure.
[b] Wave length, 290 mμ; slit width, 0.5 mm.

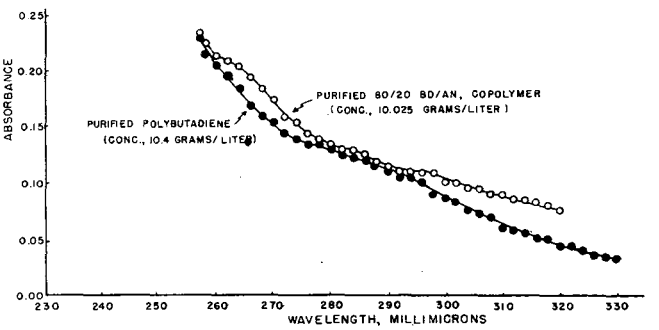


Figure 2. Absorption spectra of polybutadiene and 80/20 butadiene–acrylonitrile copolymer

Slit width, 0.5 mm.

that the analysis of combined methyl isopropenyl ketone in polymers could be accomplished in the manner described.

The transmittance curve for a butadiene–acrylonitrile copolymer (Figure 2) shows a relatively low absorbance for butadiene–acrylonitrile copolymer at 290 mμ. Table II indicates further that the average absorptivity for butadiene–acrylonitrile copolymers of variable charge ratio is 0.0097. This is practically the same value as that for butadiene polymer (0.010), so that presumably there would be no interference due to acrylonitrile polymer. The transmittance curve for 70/30 methyl isopropenyl ketone–acrylonitrile copolymer is analogous to a typical poly(methyl isopropenyl ketone) curve (see Figure 1). Based on

Table II. Absorptivity of Butadiene–Acrylonitrile Copolymers

(Wave length, 290 mμ; slit width, 0.5 mm.)

Polymer[a]	Concn., Grams/Liter	Absorbance	Absorptivity
90/10 BD/AN	7.05	0.060	0.0086
48% conversion	7.20	0.063	0.0088
	2.65	0.025	0.0095
	2.80	0.027	0.0097
80/20 BD/AN	9.30	0.085	0.0091
62.5% conversion	12.00	0.105	0.0088
	2.70	0.025	0.0093
	2.85	0.027	0.0095
70/30 BD/AN	6.45	0.070	0.0110
60.5% conversion	7.20	0.085	0.0120
	2.70	0.025	0.0093
	2.70	0.025	0.0093
60/40 BD/AN	7.35	0.085	0.0110
61.9% conversion	6.15	0.060	0.0110
	2.80	0.027	0.0097
	2.55	0.022	0.0091
Average			0.0097

[a] Prepared in bottle laboratory at charge ratios and conversions indicated. Latex was coagulated from 10% salt solution and 1% H_2SO_4. After drying for 24 hours at room temperature in vacuum oven, polymers were purified according to manner prescribed in Procedure.

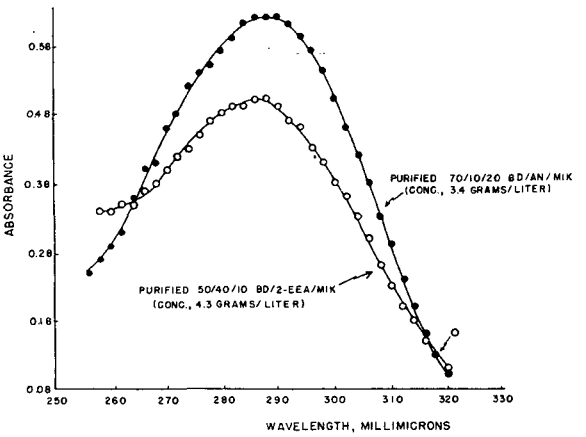


Figure 3. Absorption spectra of methyl isopropenyl ketone tripolymers

Slit width, 0.5 mm.

these findings, the method of analysis was extended to butadiene–acrylonitrile–methyl isopropenyl ketone tripolymers.

A transmittance curve for a butadiene–acrylonitrile–methyl isopropenyl ketone tripolymer is shown in Figure 3. The curve resembles the typical poly(methyl isopropenyl ketone) curve (see Figure 1); it also has a maximum inflection at 290 mμ. No apparent interference with the transmittancy of methyl isopropenyl ketone polymer on the part of acrylonitrile polymer is noted for this type of tripolymer. Table III shows the analyses of specially prepared polymers of variable charge ratios; good reproducibility is indicated. The method is rapid and reproducible to within 0.4% combined methyl isopropenyl ketone, based upon the average of quadruplicate determinations for each polymer studied.

Table III. Determination of Methyl Isopropenyl Ketone in Butadiene–Acrylonitrile–Methyl Isopropenyl Ketone Tripolymers

(Wave length, 290 mμ; slit width, 0.5 mm.)

Polymer[a]	Concn., Grams/ Liter	Absorbance	$a_{(Total)}$	MIK, %
BD/AN/MIK	4.85	0.250	0.052	7.5
85/10/5	4.16	0.200	0.048	6.8
60.6% conversion	4.15	0.205	0.050	7.1
	5.80	0.305	0.053	7.7
BD/AN/MIK	7.75	0.650	0.084	13.2
80/10/10	8.60	0.680	0.080	12.5
59.6% conversion	4.40	0.350	0.080	12.5
	4.65	0.375	0.080	12.5
BD/AN/MIK	6.50	0.870	0.134	22.1
75/10/15	7.10	0.980	0.138	22.8
56.8% conversion	8.15	1.15	0.140	23.0
	7.00	0.95	0.136	22.4
BD/AN/MIK	6.80	1.20	0.177	29.8
70/10/20	7.70	1.35	0.175	29.4
62.2% conversion	3.40	0.60	0.177	29.8
	3.35	0.61	0.182	30.6
BD/AN/MIK	8.00	1.35	0.165	27.7
60/20/20	8.10	1.35	0.166	27.9
73.4% conversion	4.00	0.67	0.167	28.0
	4.05	0.67	0.165	27.7
BD/AN/MIK	2.20	0.375	0.170	28.6
60/20/20	2.60	0.450	0.173	29.0
56.8% conversion	3.45	0.580	0.168	28.2
	2.70	0.455	0.169	28.4
BD/AN/MIK	7.80	1.22	0.157	26.2
50/30/20	9.25	1.42	0.154	25.7
81.9% conversion	3.90	0.62	0.159	26.4
	4.35	0.69	0.160	26.6
BD/AN/MIK	3.40	0.510	0.150	25.0
50/30/20	2.70	0.410	0.152	25.4
58.8% conversion	2.22	0.335	0.151	25.2
	2.50	0.370	0.151	25.2

[a] Polymers contained no PBNA and were purified according to manner prescribed in Procedure.

To demonstrate further applications of the method, a transmittance curve is shown for a butadiene–2-ethoxy ethacrylate–methyl isopropenyl ketone tripolymer (see Figure 3). An absorption maximum occurs at 290 mμ and the absence of interference is apparent. The acrylate content can be deduced from the total oxygen content of the polymer.

Table IV. Interference of Phenyl-2-naphthylamine in the Analysis of Butadiene–Methyl Isopropenyl Ketone Copolymers[a]

	Concn., Grams/ Liter	Absorbance	a^b_{Total}	Δ a Due to PBNA
90/10 BD/MIK; 58.8% Conversion				
No PBNA	2.90	0.240	0.083	
	2.90	0.235	0.081	
			0.082	
0.8% PBNA added	5.40	0.550	0.102	
	5.90	0.550	0.094	
			0.098	
				0.016
70/30 BD/MIK; 60.2% Conversion				
No PBNA	4.15	0.860	0.197	
	4.30	0.830	0.193	
			0.195	
0.7% PBNA added	4.20	0.860	0.205	
	4.55	0.880	0.195	
			0.200	0.005

[a] Polymers purified as described under Procedure.
[b] Wave length, 290 mμ; slit width, 0.5 mm.

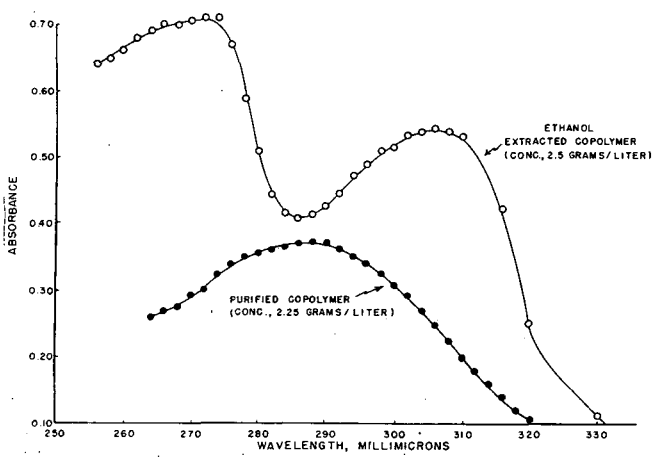


Figure 4. Absorption spectra of 80/20 butadiene–methyl isopropenyl ketone copolymers

Slit width, 0.5 mm.

During the early measurements difficulties were encountered, as shown by Figure 4. An absorption maximum was encountered at 276 mμ for 80/20 butadiene–methyl isopropenyl ketone copolymer dissolved in chloroform. This behavior indicated possible contamination of the polymer with a phenyl-2-naphthylamine (PBNA). Purification of the polymer has been accomplished by extraction with ethyl alcohol. A solution of 0.001% by weight of phenyl-2-naphthylamine in chloroform was then made up by diluting a 1% solution. Absorption maxima at 272 and 310 mμ were noted for this phenyl-2-naphthylamine solution. The presence of phenyl-2-naphthylamine would therefore interfere with readings taken at 290 mμ for analysis of combined methyl isopropenyl ketone. The polymer was subsequently purified as described under Procedure. This purification eliminated interference peaks (see Figure 4).

Further studies on interference of phenyl-2-naphthylamine,

Table V. Correction of Interference Caused by Phenyl-2-naphthylamine

(Wave length, 290 mμ, slit width, 0.5 mm.)

Polymers	Concn., Grams/ Liter	Absorbance	$a_{(Observed)}$	$a_{(PBNA)}$	$a_{(Total)}$	MIK, %
72GA2B3[a]	2.80	0.480	0.171	0.017	0.154	25.7
80/20 BD/MIK	2.70	0.470	0.173	0.017	0.156	26.1
75.9% conversion	2.90	0.495	0.171	0.017	0.154	25.7
	2.80	0.480	0.172	0.017	0.155	25.9
72GB7A4[a]	6.85	1.25	0.182	0.017	0.165	27.7
80/20 BD/MIK	6.75	1.23	0.182	0.017	0.165	27.7
58.5% conversion	6.55	1.20	0.183	0.017	0.166	27.9
	6.60	1.20	0.182	0.017	0.165	27.7
72GC2A3[a]	2.40	0.415	0.173	0.017	0.156	26.1
80/20 BD/MIK	2.30	0.410	0.172	0.017	0.155	25.9
93.6% conversion	2.75	0.475	0.173	0.017	0.156	26.1
	2.80	0.480	0.172	0.017	0.155	25.9
70G blend 1[b]	4.10	0.545	0.133	0.017	0.116	18.9
85/15 BD/MIK	4.00	0.530	0.132	0.017	0.115	18.7
62.7% conversion	5.45	0.700	0.129	0.017	0.112	18.2
	5.25	0.680	0.130	0.017	0.113	18.4
70G blend 2[b]	5.95	0.620	0.104	0.016	0.088	14.0
90/10 BD/MIK	5.90	0.610	0.103	0.016	0.087	13.8
60.9% conversion	5.90	0.610	0.103	0.016	0.087	13.8
	5.30	0.555	0.104	0.016	0.088	14.0
70G blend 3[b]	3.75	0.310	0.083	0.016	0.057	8.4
95/5 BD/MIK	4.75	0.410	0.085	0.016	0.059	8.7
61.1% conversion	3.80	0.315	0.083	0.016	0.057	8.4
	3.05	0.250	0.083	0.016	0.057	8.4
21HGA blend 1[b]	2.65	0.440	0.166	0.017	0.149	24.8
Lot 1	4.65	0.275	0.167	0.017	0.150	25.0
80/20 BD/MIK	2.65	0.445	0.168	0.017	0.151	25.2
72.3% conversion	2.05	0.350	0.171	0.017	0.154	25.7
21HGA blend 1[b]	2.25	0.375	0.166	0.017	0.149	24.8
Lot 2	3.20	0.530	0.165	0.017	0.148	24.6
80/20 BD/MIK	1.95	0.325	0.167	0.017	0.150	25.0
72.3% conversion	2.50	0.415	0.166	0.017	0.149	24.8
21HGB blend 1[c]	3.45	0.580	0.168	0.017	0.151	25.2
Lot 1	3.60	0.590	0.165	0.017	0.148	24.6
80/20 BD/MIK	4.10	0.680	0.166	0.017	0.149	24.8
59.8% conversion	3.45	0.570	0.165	0.017	0.148	24.6
21HGB blend 1[c]	4.05	0.680	0.168	0.017	0.151	25.2
Lot 2	3.40	0.580	0.170	0.017	0.153	25.5
80/20 BD/MIK	3.10	0.510	0.164	0.017	0.147	24.4
59.8% conversion	2.55	0.420	0.165	0.017	0.148	24.6
21HGB blend 1[c]	2.45	0.415	0.169	0.017	0.152	25.4
Lot 3	3.20	0.520	0.163	0.017	0.146	24.2
80/20 BD/MIK	3.10	0.510	0.165	0.017	0.148	24.6
59.8% conversion	3.15	0.515	0.163	0.017	0.146	24.2
21HGC blend 1[c]	3.55	0.550	0.155	0.017	0.138	22.8
Lot 1	2.75	0.415	0.151	0.017	0.134	22.1
80/20 BD/MIK	3.40	0.510	0.150	0.017	0.133	22.0
88.6% conversion	3.15	0.475	0.151	0.017	0.134	22.1
21HGC blend 1[c]	2.55	0.375	0.147	0.017	0.130	21.4
Lot 2	3.75	0.535	0.143	0.017	0.126	20.7
80/20 BD/MIK	2.65	0.385	0.145	0.017	0.128	21.1
88.6% conversion	3.00	0.450	0.150	0.017	0.133	22.0

[a] Purified by ethyl alcohol extraction, redissolving in chloroform, and precipitation in methanol.
[b] Raw polymers purified by double precipitation in methanol after being dissolved in chloroform. 21HGA samples retained yellow-green coloration due to incomplete removal of PBNA by usual method of purification.
[c] Purified by dissolving in 20 parts of methanol and 80 parts of chloroform and then precipitating in methanol.

shown in Table IV, indicate that phenyl-2-naphthylamine cannot be quantitatively extracted from the polymers and that apparently more of it is retained by polymers with lower ratios of methyl isopropenyl ketone–butadiene copolymer than by those of higher ratios of methyl isopropenyl ketone–butadiene copolymer. Since the data of Table IV prove the inadequacy of the method of purification for polymers containing phenyl-2-naphthylamine, it is suggested that, wherever possible, polymer coagulated from latex prior to addition of phenyl-2-naphthylamine be used for this analysis. For raw butadiene-methyl isopropenyl ketone copolymers containing phenyl-2-naphthylamine, a correction, which is based upon values shown in Table IV, may be applied. Corrections for retained phenyl-2-naphthylamine should be evaluated also for monomer compositions other than those shown in Table IV.

The data in Table V exemplify such applications. Experimental correction values were applied. More data are required before the validity of such corrections can be established. The problem of applying a correction factor for phenyl-2-naphthylamine is complicated by variations in the degree of oxidation of more phenyl-2-naphthylamine in samples representing variable polymer compositions. An exact relationship describing the dependence of nonextractable phenyl-2-naphthylamine upon monomer ratio therefore cannot easily be drawn, although a probable value for retained phenyl-2-naphthylamine may be applied, on the basis of present information.

Obviously the method for purifying the polymer is important. Dissolving the raw polymer twice in chloroform and reprecipitating with methanol at room temperature afforded good reproducibility, but was time-consuming. High temperature extractions such as ethyl alcohol extraction followed by dissolution of the polymer in chloroform invariably produced much gel and are therefore not recommended. A double precipitation method conducted at room temperature is most suitable for purifying the polymers involved.

LITERATURE CITED

(1) Central Scientific Co., Chicago, Ill., Catalog, p. 682, 1941.
(2) Weissberger, A., "Physical Methods of Organic Chemistry," Vol. II, p. 819, Interscience, New York, 1946.

RECEIVED for review May 27, 1954. Accepted December 17, 1954. Work performed as a part of the research project sponsored by the Federal Facilities Corp., Office of Synthetic Rubber, in connection with the government synthetic rubber program.

Nephelometric Determination of Sulfate Impurity in Certain Reagent Grade Salts

HUBERT J. KEILY and L. B. ROGERS

Department of Chemistry and Laboratory of Nuclear Science, Massachusetts Institute of Technology, Cambridge 39, Mass.

A rapid and precise method was desired for determination of the sulfate impurity in reagent grade calcium carbonate, sodium carbonate, and potassium chloride. A nephelometric procedure involving the addition of solid barium chloride to hydrochloric acid solutions of the sample containing 20% ethyl alcohol proved to be applicable. The salt being analyzed, its concentration, and the method of adding the reagent were investigated in detail. Prior filtration of the test solutions was shown to be essential when the sulfate concentration was below 2 p.p.m. Agreement within a range of 1.0 to 1.5 "nephelos" units was obtained at all levels of sulfate concentration (from 0.2 to approximately 10.0 p.p.m.) up to 100 nephelos units. The method of standard addition and extrapolation provided a determination of the sulfate in a particular salt sample and a calibration curve of permanent value. The procedure is general and should be easily applicable to many salts.

SMALL amounts of sulfate present as an impurity in inorganic analytical reagents are still determined in most control laboratories by the precipitation and weighing of barium sulfate. The low level of the impurity places considerable strain on the gravimetric procedure because, even when large samples are employed, the amount of barium sulfate produced is of the order of 1 mg. (*3, 30*). At best, the determination is semiquantitative, despite the fact that much time is consumed in digesting and filtering the precipitate.

A variety of volumetric methods have been proposed for the determination of sulfate. The most promising ones involve the use of tetrahydroxyquinone (*16, 22*) or rhodizonates (*2*) as indicators for barium ions. The standard chromate procedure has also been adapted to the micro scale (*9*). In addition, photometric precipitation titrations have been developed (*19, 26, 31*). Potentiometric determinations have employed the lead-amalgam electrode (*7*), bimetallic electrodes (*25*), and the ferrocyanide-ferricyanide couple (*1, 13*). A study using an amperometric titration in 30% ethyl alcohol with lead ions has been reported (*14*).

Two indirect polarographic procedures have been suggested. One of these involved the measurement of the concentration of excess lead ions after equilibrium has been reached with lead sulfate (*17*); the other (*10*) introduced preliminary reduction of the sulfate to sulfide, followed by distillation, precipitation with cadmium ion, and subsequent measurement of the cadmium ion. The sulfide has also been determined colorimetrically (*11*) by formation of methylene blue. A spectrophotometric modification of the benzidine method (*4*) has also been reported. Nephelometric, or the closely related turbidimetric, determination of barium sulfate has been frequently used for the estimation of sulfate (*5, 6, 15, 20, 21, 23, 24, 28*).

Of these methods only the measurement of the methylene blue color for sulfide or the light-scattering power of suspensions of barium sulfate seemed applicable to the levels of sulfate that were anticipated. The decision was made to try to adapt a known nephelometric procedure because this technique appeared to be faster and did not involve a separation.

Nephelometric and turbidimetric procedures have provided a variety of recommendations as to the optimum conditions for stabilizing the suspensions and improving the reproducibility of the measurements. First, the modification of the aqueous medium by adding agar, gelatin, ethyl alcohol, glycerol, and mixtures of these at various levels of concentration in water has been tried to promote the rapid attainment of a measurable and stable sol of barium sulfate. Most recently Toennies and Bakay (*28*) have recommended a 9 to 11 mixture of ethyl alcohol–dipropylene glycol at 40% by volume. As a second variable, the method of addition of the barium chloride precipitant has been shown to be an important factor affecting the reproducibility. Strikingly different ways of introducing the reagent have been suggested: Barium chloride (*5, 6, 21*) or barium acetate (*20*) crystals have been dissolved in the sulfate solution at a constant stirring rate; the solution has been seeded prior to the addition of a solution of the barium chloride (*18, 24*); and equal volumes of the reagent and sulfate solutions, which are identical in solvent composition, have been mixed by swirling (*28*).

Rather than attempt an evaluation of all the suggested techniques, the conditions which appeared to be the simplest were chosen for investigation. Ethyl alcohol at 20% by volume was selected as the organic additive for stabilizing the sol because it was readily available and because ethyl alcohol was reported by Toennies and Bakay (*28*) to result in higher intensities of scattered light than glycerol. Barium chloride crystals were used as the precipitant because they could be added conveniently from a small scoop. This eliminated the obvious disadvantage of using a reagent solution which, when mixed with the sample, necessarily diluted further the already dilute solution of sulfate. Furthermore, solutions of barium chloride have been shown to undergo an aging phenomenon which influences the crystal size of precipitated barium sulfate (*8*). If alcoholic solutions of barium chloride, used as a nephelometric reagent, were aged too long, the shape of subsequently determined curves was changed (*28*).

The following investigation was largely confined to the evaluation of these simple adaptations of the nephelometric method to the establishment of curves for determining the sulfate impurity in reagent grade calcium carbonate, sodium carbonate, and potassium chloride. These salts have maximum allowable sulfate contents, according to the recommended AMERICAN CHEMICAL SOCIETY specifications, of 0.010, 0.003, and 0.001%, respectively (*3, 30*).

EXPERIMENTAL DETAILS

Apparatus. The light scattered at right angles by the barium sulfate sols was measured with a Model 7 Coleman photonephelometer using a "null" procedure. The sensitivity of the instrument was adjusted with Coleman "nephelos" standards 10 and 38 according to a modification of the manufacturer's procedure (*12*). Using the nominal values of these standards, the instrument is at its "normal" sensitivity (1×). With the authors' particular instrument, the response to a particular standard could be increased a maximum of threefold (3×); its response could also be decreased considerably, but was never used below half its normal sensitivity (0.5×). Regardless of the instrument sensitivity employed, the results are always reported in terms of the normal sensitivity (1×).

Coleman cuvettes (No. 7-302) were used. When filled with filtered distilled water none of the cuvettes produced nephelos readings greater than 1.0 at the 3× sensitivity level.

All solutions, prior to the development of turbidities, were passed through a cellulose-ester filter disk (sold as Millipore filter by Lovell Chemical Co., Watertown, Mass.). The borosilicate glass filter holder was modified by blowing a socket joint to the mouth of the holder. This permitted the application of

gas pressure (usually only 3 to 4 pounds per square inch were required) to speed the filtration. A cylinder of nitrogen provided the pressure needed to give a rate of approximately 10 ml. per minute. Vacuum was not used to speed the filtration because of the danger of altering the alcohol content of the solution. The minimum particle size retained by the filter is not known, but optically clear solutions were invariably obtained. The filter disks became crinkled when wet by solutions which were 20% ethyl alcohol, but their usefulness did not appear to be impaired. The effect of higher concentrations of alcohol was not tested.

All pH measurements were made using a glass electrode and a Beckman Model G pH meter. Brass sieves (Cenco, commercial grade), 30-, 40-, and 80-mesh, were used to "size" crystals of reagent grade barium chloride dihydrate crystals. A small metal scoop, in which the crystals could be leveled with the edge of a spatula, consistently delivered 0.21 ± 0.01 gram of the barium chloride (30- to 40-mesh).

The 30-ml. beakers, which were used in the precipitation step, were thoroughly washed, dried, and stored in a large desiccator (no desiccant) to protect them from the dust in the air. All glassware was washed with detergent immediately after use and required no further special cleansing.

Polyethylene bottles were used for storing solutions and filtered distilled water.

Reagents. Reagent grade chemicals were used throughout. The "low-alkali" calcium carbonate from Mallinckrodt was found to be essentially sulfate-free. The concentrated (37%) hydrochloric acid from Du Pont, used for dissolving the samples of calcium and sodium carbonates, had a stated maximum limit of 0.00008% sulfur trioxide. When analyzed by the nephelometric technique, it was found to contain 0.00003% sulfur trioxide. The sulfate content of other reagents is discussed later.

The distilled water gave no test for sulfate. However, it was passed through a Millipore filter before use to remove a small but measurable turbidity.

U. S. P. absolute ethyl alcohol was obtained from U. S. Industrial Chemicals Co.

General Procedure. The general technique may be described as a three-step procedure: preparation of the sample solution, formation, and measurement of the turbidity. A 10.0-gram sample was dissolved in water or in a minimum amount of hydrochloric acid to keep the volume less than 70 ml. The pH was adjusted to 1.0 by means of hydrochloric acid and the solution transferred to a 100-ml. volumetric flask. Quantitative transfer was effected by means of pH 1.0 hydrochloric acid. Twenty milliliters of absolute ethyl alcohol were added, following which the final adjustment to volume was made with pH 1.0 hydrochloric acid. When it was desired to add a known amount of sulfate, it was added in the form of a stock solution of potassium sulfate (pH 1.0) immediately after dissolution of the sample. After a solution had been prepared, it was passed through a Millipore filter and could be stored in a capped polyethylene bottle for about 2 weeks without serious change from evaporation.

For the purpose of establishing a calibration curve, it was found convenient to prepare two solutions of 250-ml. volume which were identical in every respect except that one solution contained no added sulfate, while to the other was added sufficient sulfate to provide the highest concentration on the desired calibration curve without regard for any sulfate already in the sample. Appropriate volumes of each solution were measured conveniently from burets to make a 20.0-ml. sample for analysis.

Barium sulfate was precipitated by adding 0.42 gram of barium chloride crystals, 30- to 40-mesh, from a small scoop to a 20.0-ml. solution of sample. The crystals were dissolved with the aid of a motor-driven stirrer whose rate was reasonably constant but not exactly known (about 500 r.p.m.). In almost every case the crystals were completely dissolved within the 3-minute period to give a concentration of 0.089*M*. In the most concentrated solutions of calcium chloride, one or two small crystals occasionally remained, but they were ignored without resulting in any measurable effect.

Turbidities were usually measured from 15 to 60 minutes after precipitation. Concentrations of sulfate of 2.0 γ per ml. or less were read at two or three times the normal sensitivity of the instrument.

RESULTS

Studies Using Potassium Sulfate Alone. Three series of solutions were prepared and analyzed, each on a different day. The ranges of sulfate concentration overlapped one another and covered an over-all range of 0.2 to 10.0 γ per ml. Figure 1 shows that all of the data fell on a smooth curve and that one can clearly distinguish a difference corresponding to 0.1 γ per ml. in the region below 2.0 γ per ml.

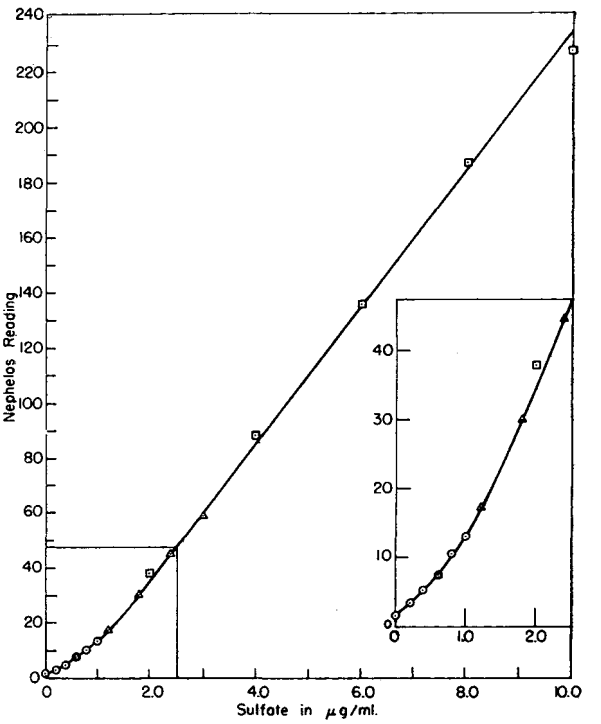


Figure 1. Curve obtained with potassium sulfate, in absence of foreign salts, using barium chloride crystals as reagent

Different symbol used for each independent series

It has been recommended (*28*) that equal volumes of sample and barium chloride solution, of the same nonaqueous content, be mixed in the precipitation step. Therefore, a comparison of these techniques was made. For this purpose, a 0.2*M* solution of barium chloride was prepared in 20% ethyl alcohol. This solution, which became slightly turbid, was aged overnight and then passed through a Millipore filter. After filtration, the reagent solution was optically clear, and remained so until it was exhausted a month later. Figure 2, a plot of typical data, shows that the results are linear, but that the points show considerable scatter.

Significantly, the use of the filtered barium chloride reagent solution resulted in readings for blank solutions which were always less than 1.0 nephelos reading unit, whereas the use of the 30- to 40-mesh solid reagent consistently produced readings of about 1.5 nephelos units. An investigation showed that this "blank" reading was larger for smaller crystal sizes of the barium chloride, being around 5.0 for 40- to 80-mesh. When 30- to 40-mesh crystals from other bottles of barium chloride were tested, the usual blank of about 1.5 units was obtained except in one case where a blank of 3.0 units was found. The insert in Figure 2 shows a comparison of measurements taken at very low sulfate concentrations using 30- to 40-mesh barium chloride crystals and a filtered 0.2*M* barium chloride solution. The deleterious effect of the larger blank for the solid barium chloride reagent disappears very rapidly, so that it does not make a significant contribution to the readings for amounts greater than about 0.4 γ per ml. of sulfate.

Sulfate Impurity in Calcium Carbonate. Analytical reagent grade calcium carbonate has a specification limit of 0.010% sulfate. Assuming that this figure would be the upper limit, a sample concentration of 0.100 gram of calcium carbonate per milliliter was selected to provide a sulfate concentration of 10.0 γ per ml. or less which, as noted in the work with potassium sulfate solutions, is the optimum working range of the nephelometer.

The "low-alkali" calcium carbonate was dissolved in a slight excess of hydrochloric acid and adjusted to pH 1.0 in the usual way. The sulfate contributed by the acid corresponded to 0.0002% sulfate in the carbonate and was small enough to be ignored. Figure 3 shows the curve obtained by adding known amounts of potassium sulfate to solutions of calcium chloride. It is obvious that the sulfate content of the carbonate was well below 0.001% and was the same order of magnitude as the blank—i.e., 0.0002%. Results from four different bottles of low-alkali calcium carbonate showed close agreement. However, other reagent grade calcium carbonates from the same company and a sample of calcium chloride dihydrate were estimated to contain amounts of sulfate between 0.001 and 0.006%.

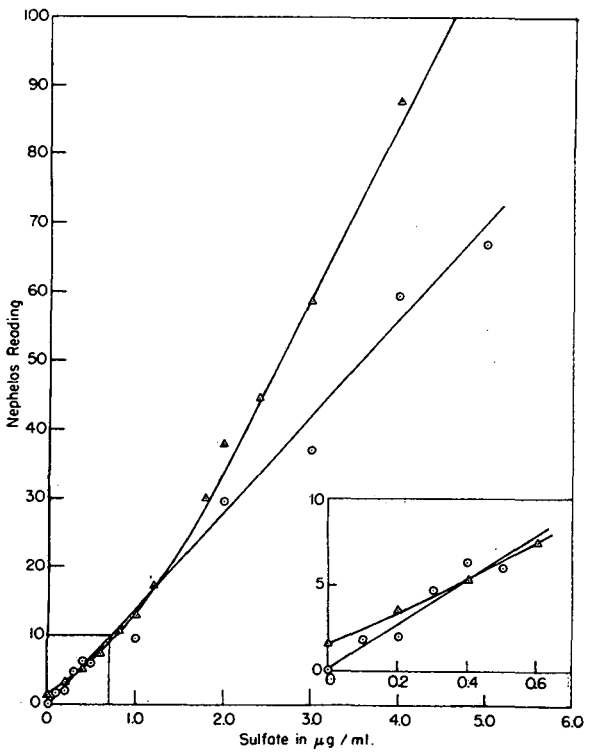


Figure 2. Comparison of curves obtained for potassium sulfate using barium chloride

△. Solid
○. Reagent solution

A number of factors were examined to determine the influence of each on the reproducibility. An important difference in the calibration curve for the potassium sulfate alone and for that obtained in the presence of 0.100 gram of calcium carbonate per milliliter (or 1.00*M* calcium chloride) was the greater-than-two-fold decrease in the sensitivity of the method due to the presence of added salt. In order to determine the effect of the sample size upon the calibration curve, one solution, prepared in the usual manner to contain 0.100 gram of calcium carbonate per ml. and 10.0 γ of sulfate per ml., was diluted with a second containing no added carbonate or sulfate to provide simultaneous changes in both the size of the sample and the sulfate concentrations. Each point on the curve in Figure 4 represents the nephelos reading that would have been obtained for a particular weight of sample, provided the sample had contained the maximum sulfate content of 0.010%. The distinct curvature can be attributed to the change in ionic strength.

Gross changes in the stirring rate used to dissolve the reagent crystals influenced the readings slightly. A very rapid stirring rate caused higher readings, while an extremely slow rate, requiring 15 minutes to dissolve all the barium chloride crystals, lowered the expected reading. A solution which would normally produce a reading of 80 nephelos units could be altered as much as 5 units in either direction by such drastic changes. However, slight variations in the stirring rate had no significant effect, nor did continued stirring at the usual rate for as long as 15 minutes after the complete dissolution of the crystals.

The turbidities for sulfate concentrations of 2.0 γ per ml. and above attained their maximum values within 5 minutes after the complete dissolution of the reagent barium chloride. The maximum difference between successive readings on the same cuvette obtained over a period of 90 minutes after the development of the turbidities was 0.5 nephelos unit. For amounts of sulfate less than 2.0 γ per ml., and especially for amounts less than 1.0 γ per ml., the development was slower, so that a period of 1 hour was allowed before recording a final measurement.

Filtration of the sulfate solutions prior to the addition of barium chloride was only necessary in analyzing samples containing 2.0 γ per ml. or less of sulfate. Below this level, readings on unfiltered solutions were often as much as 3.0 nephelos units higher (at 1×) than if the solutions had first been filtered, thereby contributing an error of about 30% at 1.0 γ per ml.

For a sulfate concentration of 10.0 γ per ml., which produced a reading of 98.0 in 20% ethyl alcohol, changes in the concentration of ethyl alcohol to 18 and to 15% did not produce a significant difference in the turbidity. Increases to 22 and 25% resulted in an increase of 1.5 nephelos units per 1% change in ethyl alcohol content. Variations from this source were made insignificant by pipetting the ethyl alcohol.

A brief study showed that a change of pH from 1.0 to 0.7 produced no significant difference in the results. However, the desirability of using a pH meter for the adjustment to pH 1.0 is discussed later.

Sulfate Impurity in Potassium Chloride. The specific maximum limit of 0.001% sulfate for reagent grade potassium chloride

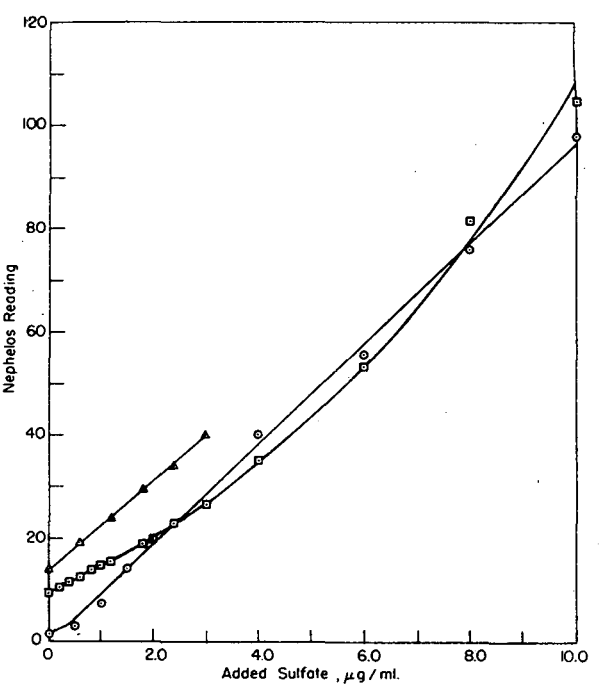


Figure 3. Curves for potassium sulfate in presence of chlorides

○. Calcium chloride
□. Potassium chloride
△. Sodium chloride

is tenfold lower than that for calcium carbonate. Hence by using a sample concentration of 0.100 gram of potassium chloride per milliliter, the maximum sulfate concentration to be expected was 1.0 γ per ml. Obviously, at this level, dust which accumulated in the solutions during their preparation had to be removed by filtration before the development of the turbidities.

Table I contains the data taken on 0.100-gram-per-ml. solutions of potassium chloride to which known amounts of sulfate had been added. A comparison of the replicates shows that, in the region of interest, the reproducibility of the method is sufficiently good to permit differences of 0.2 γ per ml. of sulfate to be distinguished. Figure 3 shows a plot of these data, together with data for higher concentrations of sulfate. Although the curve was not linear above 2.0 γ per ml. of added sulfate, its shape was reproducible. Figure 3 also shows that the potassium chloride reagent was not sulfate-free. Assuming that one can extrapolate to zero turbidity and thereby determine the sulfate concentration without added sulfate, the sulfate content of the dry reagent was estimated to be 0.0018%. Even though the slope of the lower part of the curve did not permit differences smaller than 0.2 γ per ml. to be established unambiguously, this amount corresponded to 0.0002%.

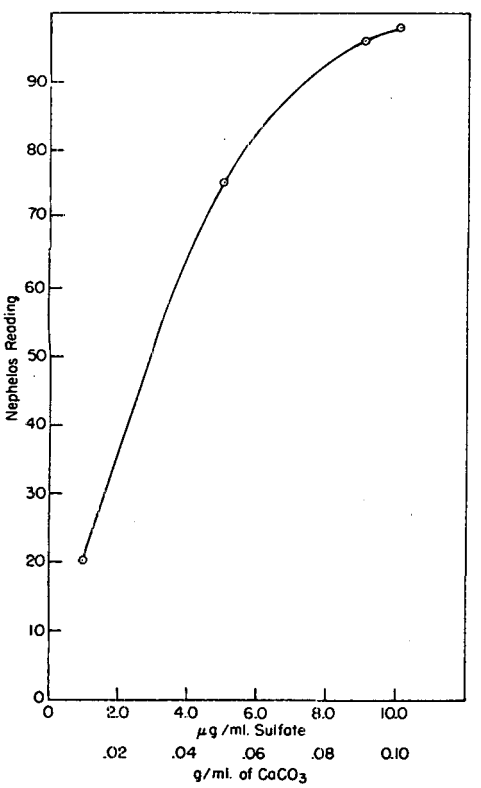


Figure 4. Effect of sample size on turbidity obtained for sample of calcium carbonate containing 0.010% sulfate

Since the extrapolated value of the original sulfate content of the potassium chloride exceeded the specified maximum level of 0.001%, two independent experiments were run to investigate the validity of the extrapolation. Four gravimetric sulfate determinations, following the recommended AMERICAN CHEMICAL SOCIETY procedure (*3*), were made on the same bottle of potassium chloride and the values 0.0014, 0.0018, 0.0014, and 0.0008% were obtained. These values indicated that the sulfate content probably exceeded the specified limit of 0.001% and that the nephelometric value of 0.0018% sulfate was reasonable.

Table I. Turbidities Developed in Four Groups of Solutions Containing 0.100 Gram per Ml. of Potassium Chloride[a]

Sulfate Added, γ/Ml.	Nephelos Readings			
	A[b]	B[b]	C[c]	D[d]
0.0	9.2	9.5	8.9	9.0
0.2	10.5	10.5	..	..
0.4	11.3	11.7	..	..
0.6	12.5	12.7	11.9	..
0.8	13.8	13.7	..	..
1.0	14.8	14.5	..	14.5

[a] Each group prepared and measured on different day.
[b] Measured at three times normal instrument sensitivity.
[c] Measured at two times normal instrument sensitivity.
[d] Measured at normal instrument sensitivity.

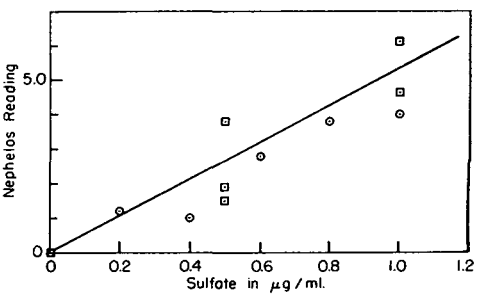


Figure 5. Check on extrapolation of curve in presence of potassium chloride in Figure 3

Different symbols represent independent sets of data

The second experiment involved the addition of micro volumes of 2.00 mg. per ml. of sulfate solution (adjusted to pH 1.0 with hydrochloric acid before being made 20% in ethyl alcohol) to 20.00-ml. volumes of a 0.100-gram-per-ml. potassium chloride solution which was 0.089*M* in barium chloride. The potassium chloride–barium chloride solution was identical in every respect to the final condition of the solutions used above in Table I, except that the sulfate impurity had previously been removed by filtration through the Millipore filter. The points in Figure 5 are the results obtained in two different series, while the line about which the points are scattered is the extrapolated portion of the curve in Figure 3. The extrapolation, therefore, appeared to be valid.

Sulfate Impurity in Sodium Carbonate. Reagent grade sodium carbonate has a specified maximum limit for sulfate impurity content of 0.003%, which is intermediate between the limits of the two salts already discussed. A sample concentration of 0.100 gram per ml. of sodium carbonate would yield a sulfate concentration of 3.0 γ per ml. if the carbonate contained the exact limit for sulfate.

The samples of sodium carbonate were prepared for analysis in the same way as those of calcium carbonate. Figure 3 shows the curve obtained when known amounts of sulfate were added to solutions of this salt. On this curve, the concentrations for which only a single point is shown were actually duplicates which agreed to within 0.3 nephelos unit.

As in the case of the potassium chloride, the sodium carbonate was not initially sulfate-free. An extrapolation of the curve, which was strictly linear over the region investigated, indicated that the sulfate content of the reagent was 0.0015%.

DISCUSSION

During the course of the work done with the calcium chloride solutions, a factorial experiment was undertaken to show the main effects and interactions of the following three variables at two levels of each: the sample size (0.0667 and 0.100 gram of calcium carbonate per ml.), the acidity (pH 0.83 and 1.00), and

the added sulfate content (2.0 and 10.0 γ of sulfate per ml.). The only significant result found, with the obvious exception of the main sulfate effect, was an interaction between the sample size and the sulfate content. This interaction is also evident in Figure 4. However, this interaction actually was confounded with the rate of dissolution of the barium chloride crystals. The full 3 minutes were required to dissolve the barium chloride in the most concentrated calcium chloride solutions, whereas they dissolved more rapidly in the more dilute solutions. (The crystals dissolved most rapidly—in approximately 1 minute—in solutions containing only potassium sulfate.) It was not possible, with the authors' motor, to regulate the speed of the stirrer with sufficient accuracy to provide a constant dissolution rate for all conditions.

The pH values discussed in this paper were those obtained with a glass electrode, and not those calculated from the known excess of acid. Observations showed that the pH of a concentrated calcium chloride solution was altered several pH units by the addition of such small amounts of hydrochloric acid that the changes could not be accounted for on the basis of the acid concentration. For example, the pH of 50 ml. of a 3.0*M* solution of calcium chloride at a pH of 8.1 was changed to 0.6 by the addition of 0.05 ml. of concentrated (37%) hydrochloric acid. (The addition of 0.20 ml. produced a pH less than zero.) Furthermore, the dilution of a 2.0*M* calcium chloride solution of pH 1.00, with a hydrochloric acid solution of pH 1.00, resulted in a decrease in pH of the resulting diluted solution. This change in pH reached a maximum of 0.17 pH unit at a calcium chloride concentration of 1.5*M*, and, upon extreme dilution, returned to a value of 1.00. This pH effect, which would be encountered during the preparation of solutions, was shown in the factorial experiment not to be significant.

In preliminary studies using reagent solutions of barium chloride, an attempt was made to use the dipropylene glycol–ethyl alcohol mixture recommended by Toennies and Bakay (*28*), but exceptionally high blanks were encountered. When ethyl alcohol alone was used, it was found to be satisfactory. However, as soon as a shift was made from pint bottles to 5-gallon metal drums (from the same supplier), larger blanks were encountered. The difficulty in both cases may have arisen either from sulfate impurity or from suspended foreign matter in the solvent. Hence, the solvent may require further treatment in order for one investigator to reproduce his own work or that of another.

The amount of barium chloride employed for precipitation was not at all critical. However, if one fourth the usual amount was employed, the turbidities developed much more slowly; they reached maximum turbidities that were at least 25% less than those usually obtained; and they lacked the usual stability at their maximum values.

It is unquestionably desirable that a calibration curve be linear, but this is certainly not a requirement, provided the measurements can be reproduced with precision. Using filtered solutions, independently prepared from the same bottle of dry salt and measured on different days, agreement within a range of 1.0 to 1.5 nephelos units at the normal instrument sensitivity was obtained at all levels of sulfate concentration up to 100 nephelos units. That portion of the variability which can be attributed to the instrument alone has been estimated to have a standard deviation of 0.28 nephelos unit at normal sensitivity (*12*). Without doubt, the reproducibility attainable for sulfate concentrations which give readings below 10 nephelos units was made possible largely by the removal of extraneous dust by filtration of the solutions prior to development of the turbidities.

The shape of a calibration curve will clearly be a function of the ionic strength of the solution, the specific salt in the solution, and the method of adding the barium. For amounts of sulfate less than 5 γ per ml., it was necessary to determine a separate curve for each salt.

Although a sample concentration of 0.100 gram per ml. was used for each salt in this study, Figure 4 suggests that smaller concentrations might be used without suffering a proportional decrease in sensitivity. For example, decreasing the concentration of calcium carbonate to 0.050 gram per ml. caused a drop of only 25% in the reading. There is also the possibility of increasing the sensitivity somewhat by working at a level of 30% alcohol (*27*, *28*).

The recommended AMERICAN CHEMICAL SOCIETY limit for sulfate impurity in reagent grade potassium chloride is 0.001% (*30*), which means that a 25.0-gram sample containing the limiting amount will produce 0.6 mg. of barium sulfate. Not only is such an amount difficult to collect completely but, with a realistic estimation of $\pm$0.2 mg. error in weighing, the probable accuracy and precision also leave much to be desired. It appears that there would be not only a saving in the amount of time required for an analysis, but also an improvement in the reliability of the result through substituting a nephelometric procedure for the gravimetric.

Ideally, calibration curves should be prepared from sulfate-free salts. However, in the present investigation only the "low-alkali" calcium carbonate was essentially sulfate-free. Gravimetric estimations of the sulfate content of this salt were fruitless, yielding no weighable amounts of precipitate. Thus, the curve in the presence of calcium chloride (see Figure 3), obtained by dissolving the "low-alkali" carbonate in hydrochloric acid and adding known amounts of sulfate, represents a direct calibration of sulfate impurity. The slight turbidity found in the solution to which no sulfate was added was due almost entirely to the dust on the barium chloride crystals. In the case of potassium chloride, the salt used for establishing the curve was not initially sulfate-free. However, its sulfate content was determined by extrapolation, and this value was checked gravimetrically. The validity of the extrapolation was subjected to further investigation by a method of standard addition to a solution which had been freed from sulfate by prior precipitation and filtration (Figure 5). Having established the initial sulfate content of the potassium chloride used, the curve, including the extrapolated region, represented a complete calibration curve. In the case of sodium chloride, the initial content of the salt was also determined by extrapolation on the assumption that the curve, at lower concentrations of sulfate, would be linear like the one for potassium chloride.

Recently, Turnbull (*29*) has studied the kinetics of precipitation of barium sulfate. This work suggests that nucleation is essentially complete before thorough mixing of reagent solutions can be accomplished, and that the number of precipitation nuclei formed in any particular case depends not only upon the gross concentrations of the reagents but also upon uncontrollable differences in the supersaturation of small local regions within the solution during the mixing process. Subsequent growth of the precipitation nuclei then produces measurable turbidities. Since the reproducibility of the light-scattering property of replicate determinations depends largely upon the number of nuclei initially formed, the use of solid barium chloride crystals apparently provided regions which were more uniformly supersaturated than those obtained using reagent solutions. Nucleation probably occurred at the solution-crystal interface where supersaturation of the barium sulfate was controlled by the rate of dissolution of the barium chloride.

SUMMARY

The sulfate impurity in reagent grade calcium carbonate, sodium carbonate, and potassium chloride (with maximum specified impurities of 0.010, 0.003, and 0.001%, respectively) has been estimated by the nephelometric measurement of barium sulfate sols. The sulfate content of each reagent has been determined by a method involving standard addition and extrap-

olation. This procedure not only permitted the estimation of the sulfate content of a salt, but also provided a permanent calibration curve. At the high salt concentrations used, the shapes of the calibration curves depended upon the specific salt under investigation and its concentration. At a concentration of 0.1 gram per ml., differences of 0.0002% sulfate were detected unambiguously. The range of sulfate content to which the nephelometric procedure has been applied is from 0.0002 to 0.010%. Because of the dust adhering to the crystal surfaces of the reagent, sulfate estimations below 0.0004% required a blank correction which could be safely ignored at higher sulfate levels.

The reproducibility of the nephelometric procedure is greatly enhanced by the use of barium chloride reagent in a solid form. Ethyl alcohol, at a level of 20% by volume, was used to stabilize the sols, and still permitted a high concentration of inorganic salt. Prior filtration of the sample solution was required when the sulfate concentration was below 2.0 γ per ml.

Although only three salts have been investigated, the general technique should be easily adapted to the determination of the sulfate impurity in a large number of other inorganic salts.

ACKNOWLEDGMENT

The authors wish to acknowledge support of this study during 1952–53 by Mallinckrodt Chemical Works, and during 1953–54 by the Atomic Energy Commission.

LITERATURE CITED

(1) Agasyan, P. K., *Vestnik Moskov. Univ.*, **8** (No. 8), *Ser. Fiz. Mat. i Estestven. Nauk.*, No. **5**, 121–4 (1953).
(2) Alicino, J. F., ANAL. CHEM., **20**, 85 (1948).
(3) AMERICAN CHEMICAL SOCIETY, Washington, D. C., "Reagent Chemicals, AMERICAN CHEMICAL SOCIETY Specifications, 1950."
(4) Andersen, L., *Acta Chem. Scand.*, 7, 689–92 (1953).
(5) Boyars, C., ANAL. CHEM., **20**, 87 (1948).
(6) Burke, O. W., Jr., Starr, C. E., Jr., and Tuemmler, F. D., "Light Hydrocarbon Analysis," pp. 371–82, Reinhold, New York, 1951.
(7) Dutta, S. K., and Ghosh, B. N., *J. Indian Chem. Soc.*, **28**, 383–9 (1951).
(8) Fischer, R. B., and Rhinehammer, T. B., ANAL. CHEM., **25**, 1547 (1953).
(9) Geilmann, W., and Bretschneider, G., *Z. anal. Chem.*, **139**, 412–23 (1953).
(10) Horton, A. D., and Thomason, P. F., ANAL. CHEM., **23**, 1859–60 (1951).
(11) Johnson, C. M., and Nishita, H., *Ibid.*, **24**, 736–42 (1952).
(12) Keily, H. J., and Rogers, L. B., *Ibid.*, **27**, 459 (1955).
(13) Kolthoff, I. M., and Furman, N. H., "Potentiometric Titrations," 2nd ed., p. 331, Wiley, New York, 1947.
(14) Kolthoff, I. M., and Pan, Y. D., *J. Am. Chem. Soc.*, **62**, 3332–5 (1940).
(15) Little, H. J. (unpublished work made available to Fisher Scientific Co.), *Chem. Eng. News*, **30**, L39, 5198 (1952).
(16) Ogg, C. L., Willits, C. O., and Cooper, F. J., ANAL. CHEM., **20**, 83–4 (1948).
(17) Ohlweiler, O. A., *Anal. Chim. Acta*, **9**, 476–88 (1953).
(18) Reimers, F., and Gottlieb, K. R., *Acta Pharm. Intern.*, **1**, 55–70 (1950).
(19) Ringbom, W., *Z. anal. Chem.*, **122**, 263–79 (1941).
(20) Rubia Pacheco, J. de la, and Blasco Lopez-Rubio, F., *Inform. quím. anal. (Madrid)*, **5**, 1–6 (1951).
(21) Rudy, R. B., *J. Research Natl. Bur. Standards*, **16**, 555–61 (1936).
(22) Siegfriedt, R. K., Wiberley, J. S., and Moore, R. W., ANAL. CHEM., **23**, 1008–11 (1951).
(23) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," 3rd ed., Vol. **II**, p. 769, Van Nostrand, New York, 1949.
(24) Steinbergs, A., *Analyst*, **78**, 47–53 (1953); *J. Australian Inst. Agr. Sci.*, **17**, 155 (1951).
(25) Takagi, K., Shimizu, Y., and Nishino, S., *J. Electrochem. Soc., Japan*, **18**, 123–5 (1950).
(26) Takagi, K., and Yamada, M., *Ibid.*, **18**, 9–12 (1950).
(27) Tananaev, I. V., and Rudnev, N. A., *Zhur. Anal. Khim.*, **5**, 8209 (1950).
(28) Toennies, G., and Bakay, B., ANAL. CHEM., **25**, 160–5 (1953).
(29) Turnbull, D., *Acta Metallurgica*, **1**, 684–91 (1953).
(30) Wichers, E., Ashley, S. E. Q., Butler, A. Q., Clarke, B. L., Collins, W. D., Eisenhauer, F. S., Osborn, R. A., Ross, J. F., and Wolf, J. P., ANAL. CHEM., **25**, 365–80 (1953).
(31) Wickbold, R., *Angew. Chem.*, **65**, 159–61 (1953).

RECEIVED for review September 7, 1954. Accepted January 4, 1955.

New Solvent System for Separating Monocarboxylic Acids (C_2 to C_{16}) and Dicarboxylic Acids (C_2 to C_{22})

VLADIMIR ZBINOVSKY

Wisconsin Alumni Research Foundation, Madison, Wis.

Silicic acid columns capable of quantitatively separating all the saturated fatty acids from C_2 to C_{14} have been developed. These columns also separated fatty acids differing by two carbon atoms in the range C_{13} to C_{16}, and all the dicarboxylic acids from C_2 to C_{22}. The solvents are methyl Cellosolve–water as internal phase, and Skellysolve B, *n*-butyl ether, or mixtures of these as external phase. The method is relatively rapid and should be useful in the chemistry of natural products and pharmaceuticals, in the oxidative determination of the structure of unsaturated acids, and in biological problems in the field of fat metabolism.

IN THE course of a study of the effect of partial hydrogenation on the position of the double bonds of unsaturated fatty acids it became necessary to determine the mono- and dicarboxylic acids produced by oxidative cleavage. The classical methods were deemed too cumbersome and the desirability of a chromatographic separation of the resulting acids on a single column became evident.

Existing methods (*1–3, 5–9*) are not satisfactory over the entire range of acids which might be encountered. The usual method (*1*) of separating dicarboxylic acids from monocarboxylic acids by extracting the mono- acids into petroleum ether did not give clean separations of small amounts of acids. Therefore it was desired to improve and extend methods of determining the acids of both series without a preliminary separation. The method of Higuchi and coworkers (*3*) was found to give the simplest and most effective separation of dicarboxylic acids from C_4 to C_{10}; the method of Begemann (*1*), also used by Boelhouwer (*2*), did not give sharp separations of C_{11} to C_{13} dicarboxylic acids.

In this study it has been possible to obtain separations in the dicarboxylic series from C_2 to C_{22}, the highest acid investigated. Further work has been initiated on acids higher than C_{22}; the upper limit of separation has not been reached. All the adjacent straight-chain fatty acids from C_2 to C_{14} can be separated on one column if necessary, although shorter columns are more con-

venient for mixtures of short-chain acids, which are eluted rather slowly.

APPARATUS

A diagram of the jacketed column and solvent reservoir, through which circulates water at a constant temperature, is shown in Figure 1. A small stopcock, *J*, was used to prevent accumulation of solvent above the delivery stopcock. Air pressure was applied to the reservoir and column through a three-way stopcock, *A*. A drop and time counting fraction collector (Research Equipment and Service, Chicago, Ill.) was used.

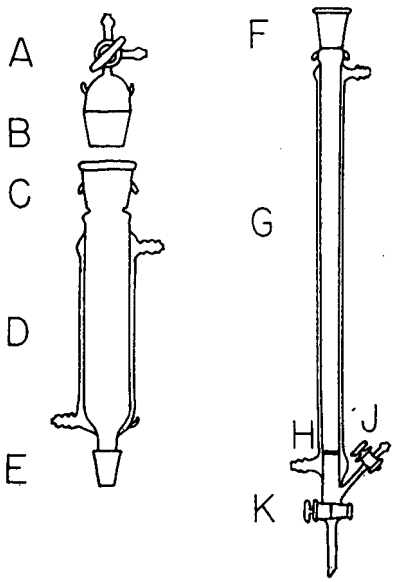


Figure 1. Jacketed column and solvent reservoir

A. Three-way stopcock
B, C. Male and female 55/50 joint
D. 35 × 6 Cm. jacketed tube
E, F. Male and female 19/38 joint
G. 55 × 1.0 Cm. jacketed column
H. Medium porosity Alundum disk
K. Delivery stopcock

Constant-pressure regulator and air filter, 0 to 15 pounds per square inch (Moore Products Co., Philadelphia, Pa.), attached through a soda-lime tube to the column cap.

Gilmont ultramicroburet graduated to 0.001 ml. (Emil Greiner Co.). Two-milliliter Cornwall pipetting unit (A. S. Aloe Co., St. Louis, Mo.).

Blotting paper disks (*10*).

Packing tube, 9.5 mm. (in outside diameter) × 60 cm. borosilicate glass tube sealed and flattened at one end, with a 1-mm. hole drilled through the center of the flattened end.

Thermostat, 25° C., for cooling the jacketed column and for equilibrating the solvents if the room temperature varies by more than ±2° C.

A wire stirrer consisting of a 5-cm.-long, 22-gage Nichrome wire attached at one end to a 60-cm.-long, 3-mm.-wide aluminum rod. Four millimeters of the other end of the wire were flattened and bent at a right angle.

MATERIALS AND REAGENTS

Silicic acid (Mallinckrodt No. 2847, analytical reagent grade), 100-mesh, dried for 16 hours at 110° C.

Skellysolve B, technical, distilled over sodium hydroxide pellets, boiling point, 66° to 67° C. (Skelly Oil Co., Kansas City, Mo.)

n-Butyl ether (Mathieson), practical, freed from peroxides by shaking with ferrous sulfate solution and distilled over sodium hydroxide pellets, boiling point, 139° to 140° C. Stored in refrigerator.

Methyl Cellosolve (ethylene glycol monomethyl ether). Distilled over sodium hydroxide pellets, boiling point, 123° to 124° C. (Carbide and Carbon Chemicals Co., Chicago, Ill.).

Phenol red indicator stock solution, 0.05%, in 50% aqueous methanol, neutralized to pH 7 with dilute sodium hydroxide solution.

Bromocresol green indicator (BCG), 100 mg. dissolved in 200 ml. of 50% aqueous methanol.

Concentrated ammonium hydroxide.

Tank nitrogen.

Methanol, refined (Merck), distilled over calcium carbonate.

Acids. The C_2 to C_{18} monocarboxylic acids (Eastman Kodak, White Label) were purified by fractional distillation or recrystallization until free from chromatographically detectable impurities. The C_2 to C_{22} dicarboxylic acids were obtained commercially, from private sources, or by synthesis in some cases. Purity was determined by chromatographic means.

COLUMN TECHNIQUES

Internal Phase. Methyl Cellosolve–water (9 to 1 volume/volume) was equilibrated with the developing solvents (Skellysolve B, *n*-butyl ether, and mixtures of both) in the ratio of 10 parts of solvent to 1 part of internal phase. When developing solvents are shaken with the internal phase, part of the internal phase dissolves in the developing solvent. This volume decrease of internal phase is shown in Table I and should be taken into consideration when stock solvents are prepared.

Table I. Volume Changes in Internal Phase after Equilibration

Solvent	Internal Phase, Ml.		Per Cent of Internal Phase Remaining
	Initial	Final	
Skellysolve B	20	17	85
n-Butyl ether	20	10	50
Skellysolve B–*n*-butyl ether (1:1)	20	13.5	67

It is important to calculate the amounts of ammonium hydroxide and bromocresol green required on the basis of the final volume of equilibrated internal phase, as the proper amounts must be determined for any given silicic acid and also for the amount of solvent prepared. A solution containing 0.012 ml. of concentrated ammonium hydroxide and 0.2 ml. of bromocresol green per 5 ml. of the equilibrated internal phase was required to give the proper blue-green tint to 6 grams of the silicic acid. The organic acids will appear as bright yellow bands on a blue-green background, if the proper amount of ammonium hydroxide is used.

External Phases (Developing Solvents). SKELLYSOLVE B. Ten volumes of Skellysolve B, 1 volume of internal phase, and the calculated volume of ammonium hydroxide and bromocresol green were shaken in a separatory funnel and allowed to stand at 25° C. for 1 hour.

n-BUTYL ETHER. *n*-Butyl ether was equilibrated in the same ratio as for Skellysolve B, making allowances for the smaller final volume of internal phase obtained, particularly if a column of larger size than 6 grams is to be prepared.

MIXTURES OF SKELLYSOLVE B AND *n*-BUTYL ETHER. These are prepared similarly, using the required volumes of fresh solvents. The solvents should first be mixed in the desired ratio and then equilibrated with methyl Cellosolve. Attempts to use solvents which had been previously equilibrated separately and then mixed always failed.

The reference column contained 6 grams of silicic acid and 5 ml. of the equilibrated methyl Cellosolve phase. This ratio was constant for any weight of silicic acid. As a specific example, 30 ml. of methyl Cellosolve were equilibrated with 300 ml. of Skellysolve B, 0.06 ml. of concentrated ammonium hydroxide, and 1 ml. of bromocresol green solution. The internal phase decreased to 25 ml., 5 ml. of which were added to 6 grams of silicic acid.

Packing the Column. Five milliliters of the blue internal phase were pipetted into a 30-ml. beaker. Silicic acid, 6 grams, was quickly weighed into the beaker, which was then cooled in tap water. The contents were thoroughly mixed with a glass rod, pressing out lumps against the wall until homogeneous. The dry powder was transferred to a 500-ml. porcelain mortar and further ground with a pestle. Developing solvent was added in three 10-ml. portions, with grinding after each addition, until a uniform suspension was obtained. The remainder of the solvent (about 240 ml.) was then added and well mixed with the silicic acid slurry. Air bubbles were removed by gentle stirring, and about 10 ml. of the supernatant fluid were pipetted into the column from a 10-ml. volumetric pipet provided with a rubber bulb. About 10 ml. of the slurry were introduced under the

surface of the solvent layer in the column. The silicic acid should fall without adhering to the walls. Tapping the column or stirring with the wire stirrer is recommended in order to release air bubbles from the column. A pressure of 2 pounds per square inch was applied until no further packing of the silicic acid could be noted. The remainder of the silicic acid slurry was added portionwise in the same manner. The surface should be uniform and horizontal. A tightly fitting blotting paper disk (*10*) was firmly pressed on top of the silicic acid with the glass packing tube, using approximately 10 pounds per square inch of pressure. If any silicic acid escapes above the disk, a slightly larger disk should be used.

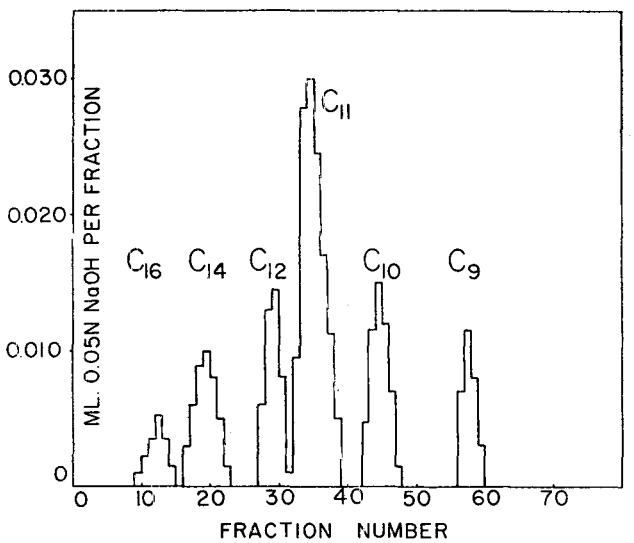


Figure 2. Separation of C_9 to C_{16} monocarboxylic acids (2.8 mg. of total acids) on 15-gram silicic acid column

Fractions 1 to 25, 0.25 ml.; 26 to 56, 0.5 ml.; and 57 to 60, 1.0 ml. Solvent, Skellysolve B

Sample Introduction. In all cases, the sample was introduced in 0.25 ml. of equilibrated external phase (developing solvent) containing a trace of Sudan III dye as marker for the solvent front. The maximum sample size used was approximately 3.5 mg. per gram of silicic acid. Where the acids were not sufficiently soluble in the external phase, 0.25 ml. of equilibrated internal phase was substituted. This did not affect the separations obtained, as it constituted only 5% of the internal phase already present. If necessary, the sample mixture and solvent could be heated to ca. 50° C. to dissolve the acids, then cooled to 25° before introduction of the sample. If crystallization occurs, it indicates that smaller amounts of acids should have been used.

The sample was placed on the still-moist disk with a pipet and allowed to penetrate the column under slight pressure. The surface of the disk was washed with three or four successive 0.1-ml. portions of developing solvent. The solvent reservoir was attached to the column, and the remainder of the developing solvent was poured in, without regard to traces of silicic acid, which at this point do not interfere with the operation of the column. A pressure of 2 pounds per square inch was applied, and development of the column was begun.

Column Development. The order of solvent addition was Skellysolve B, combinations of Skellysolve B and *n*-butyl ether, and finally *n*-butyl ether alone, according to the nature and chain length of the acids present. Collection of fractions was begun simultaneously with the introduction of the sample. Where peaks were expected to lie close together, 0.25-ml. fractions were collected. Where peaks were widely separated, the fractions could be as large as 1.0 ml. When pressures of 2 to 4 pounds per square inch were used, flow rates ranged from 0.08 to 0.2 ml. per minute. Time required for a run ranged from 1 to 24 hours. For example, to separate C_{16} from C_{14} (monocarboxylic acids) requires about 1.5 hours; separation of 15 acids on a single column can be done in about 20 hours (Figure 4).

Titration of Fractions. To 500 ml. of distilled methanol and 10 ml. of phenol red stock solution, sufficient dilute sodium hydroxide was added to give a negligible titration blank. Each fraction received 2 ml. of this indicator solution from a 2-ml. Cornwall pipetting unit. Titrations were performed with 0.05*N* aqueous sodium hydroxide solution delivered from a Gilmont ultramicroburet, while the fractions were stirred with a stream of nitrogen bubbles.

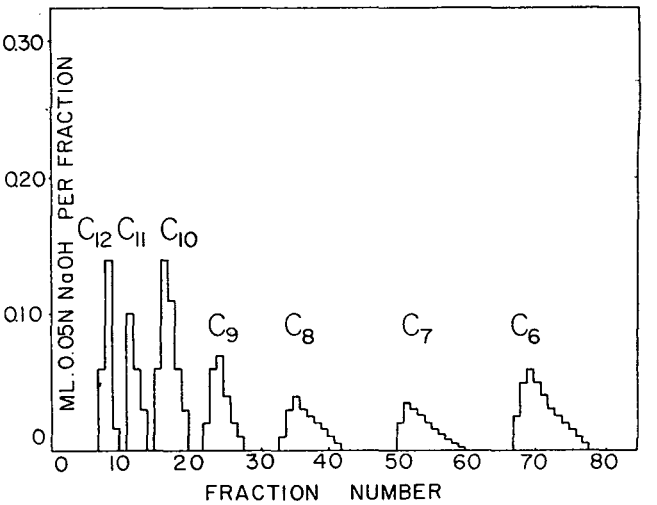


Figure 3. Separation of C_6 to C_{12} dicarboxylic acids (6.9 mg. of total acids) on 2-gram silicic acid column

Fractions 1 to 66, 1.0 ml.; and 67 to 78, 2.0 ml. Solvent, *n*-butyl ether

RESULTS

Recoveries. Table II shows the recoveries (97 to 103%) which were obtained by using samples from 0.2 to 3.5 mg. of acids per gram of silicic acid. These were determined largely from the graphs presented in Figures 2 to 5. Those acids for which recovery data are not given lie in the region where reported procedures are satisfactory and were not important for the purpose of this work. However, these acids were separated on described columns and gave narrow, well-separated bands.

Table II. Recovery of Mono- and Dicarboxylic Acids

	Monocarboxylic			Dicarboxylic		
Acids	Calcd., mg.	Found, mg.	Recovery, %	Calcd., mg.	Found, mg.	Recovery, %
C_{22}	...	...	...	0.286	0.287	100.3
C_{20}	...	...	...	0.313	0.304	97.4
C_{18}	0.502	0.487	96.9	0.215	0.211	98.2
C_{16}	0.212	0.214	100.9	...	...	...
C_{14}	0.494	0.480	97.2	0.441	0.445	100.7
C_{13}	...	...	...	0.179	0.177	98.9
C_{12}	0.193	0.190	98.5	0.238	0.244	102.9
C_{11}	...	...	...	0.133	0.135	101.5
C_{10}	0.512	0.508	99.4	0.207	0.207	100.0
C_9	0.246	0.246	100.0	1.059	1.036	97.9
C_8	0.193	0.195	101.0	0.792	0.806	101.7
C_7	0.205	0.202	98.6	0.708	0.694	98.0
C_6	0.158	0.162	102.5	1.110	1.113	100.2
C_5	0.232	0.234	100.8			
C_4	0.203	0.204	100.4			

SEPARATIONS OBTAINED

Figure 2 shows the results of a typical analysis of a mixture of known monocarboxylic acids. For the monocarboxylic acids C_9 to C_{16} a 15-gram column (46 cm.) was required. This column was prepared in the same way as the standard 6-gram column, but was packed in two equal segments separated by a blotting paper disk. Such segmented columns were found to give more uniform bands than could be obtained from an unsegmented column of the same weight. When a 15-gram column was used, the initial flow rate was 0.6 ml. per minute, which was raised at fraction 26 to 1.2 ml. per minute by increasing the air pressure.

The separation of lower and intermediate dicarboxylic acids is illustrated in Figure 3. A 2-gram column (6.5 cm.), using *n*-

butyl ether as external phase, was chosen. Although the separations were clear-cut, the C_6 to C_8 acids showed considerable tailing, and a 1.5-gram column could have been used to narrow the bands.

The separation of seven monocarboxylic and eight dicarboxylic acids on a single 6-gram column is shown in Figure 4. The acid mixture was introduced in 0.25 ml. of equilibrated *n*-butyl ether. Development with Skellysolve B eluted the monocarboxylic acids, leaving the dicarboxylic acids at the top of the column as a single yellow band. Further development with Skellysolve B–*n*-butyl ether (1 to 1) and with *n*-butyl ether slowly eluted the dicarboxylic acids. The C_{15}, C_{16}, and C_{17} acids were not available, but the graph shows that there is ample space for the complete separation of these acids. The run was completed in 20 hours.

Figure 5, *a*, shows the separation of a mono- and a dicarboxylic acid having the same chain length, and is an example of the versatility of this system.

The previous methods cited above usually gave evidence of incomplete resolutions when pairs of adjacent acids were present in widely different ratios. Figure 5, *b*, shows the separation of C_{10} and C_9 dicarboxylic acids where the ratio was 10 to 1. The recoveries were 98 and 104%, respectively.

DISCUSSION

Advantages of Method. The column support consisted of commercially available silicic acid, which needed only to be dried before use. Air bubbles were easily removed, and the column was quickly prepared and very uniform. As many as five runs were made using the same column, provided that equilibrium conditions were maintained. However, the separations reported here were made on freshly prepared columns. Such reuse simplifies the procedure where many similar runs are necessary. The column was stable over a range of ±5° C. during the runs, although it was preferable to work at a constant temperature using jacketed columns. Any combination of monocarboxylic acids from C_2 to C_{14}, and the dicarboxylic acids from C_2 to C_{22}, can be separated. The mono- and dicarboxylic acids present in a mixture can be separated on one column. All the acids tested, either singly or in mixtures, gave satisfactory recoveries, as shown in Table II. Both monocarboxylic and dicarboxylic acids (C_2 to C_{10}) are visible on the column. Any range of these acids can be separated on one column of sufficient length. However, the time required would be excessive, and the peaks would be flattened. Table III gives the recommended weights of silicic acid for the separation of any combination of the acids in a minimum time. The developing solvents contain no alcohols, eliminating loss of acids through esterification (*4*). The solvents are readily available and reasonably inexpensive.

Table III. Recommended Solvents and Amounts of Silicic Acid for Different Types and Chain Length of Acids

Monocarboxylic Acids	Silicic Acid, Grams	Solvents
C_2 to C_8	6	Skellysolve B, followed by *n*-butyl ether
C_9 to C_{16}	15	Skellysolve B
Dicarboxylic		
C_2 to C_5	0.5–1.0	*n*-Butyl ether
C_6 to C_{12}	2	*n*-Butyl ether
C_{10} to C_{22}	6	Skellysolve B, followed by Skellysolve B–*n*-butyl ether (1:1), and *n*-butyl ether alone

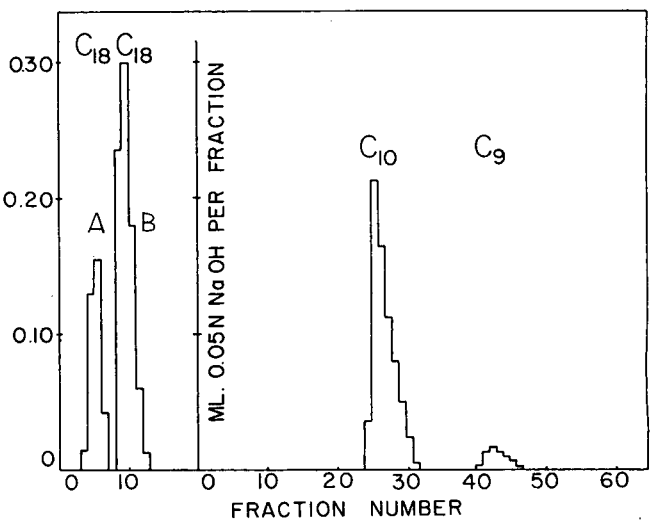


Figure 5,*a*(*left*). Separation of C_{18} monocarboxylic acid (*A*) and C_{18} dicarboxylic acid (*B*) on 6-gram silicic acid column

Fractions 1 to 3, 2.0 ml.; and 3 to 13, 0.5 ml. Solvent, *n*-butyl ether
12.4 mg. of total acids used

Figure 5,*b*(*right*). Recovery of C_{10} and C_9 dicarboxylic acids (ratio 10:1, 4.85 mg. of total acids) on 6-gram silicic acid column

Fractions 1 to 48, 2.0 ml.
Solvent, *n*-butyl ether

Factors Affecting Separations. Attempts were made to increase the speed of elution of the lower mono- and dicarboxylic acids (C_2 to C_{12}) on a 6-gram silicic acid column by varying the temperature, the water content of the internal phase, and the developing solvent.

TEMPERATURE. A column equilibrated and run at higher temperatures (50° C.) could be used to separate the C_2 to C_6 monocarboxylic acids or the C_6 to C_{10} dicarboxylic acids, with a saving in time of about 30%. At 50° C., 6 hours are required; at 25° C., 9 hours are required.

WATER CONTENT. Increasing the water content of the internal phase from 10 to 50% caused the C_8 to C_{14} monocarboxylic acids to elute in one peak, but the valley between C_4 and C_6 mono acids was four times as wide, and C_4 was eluted only in 3

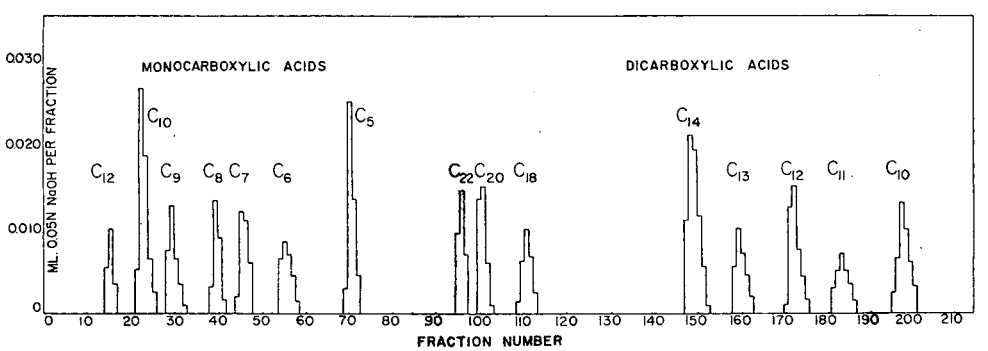


Figure 4. Separation of monocarboxylic acids (1.8 mg. of total acids) and dicarboxylic acids (2.0 mg. of total acids) on 6-gram silicic acid column

Fractions 1 to 39, 0.5 ml.; 40 to 69, 1.0 ml.; 70 to 73, 2.0 ml.; 74 to 171, 0.5 ml.; and 172 to 202, 1.0 ml.
Solvents, Fraction 1 to 73, Skellysolve B; 74 to 127, Skellysolve B–*n*-butyl ether mixture (1:1); 128 to 202, *n*-butyl ether

hours. Substitution of 0.5*N* or 2.0*N* sulfuric acid for the 10% water of the internal phase was of no advantage in separating either mono- or dicarboxylic acids.

SOLVENT. Substituting Skellysolve E (boiling point, 118° to 123° C.) for Skellysolve B caused an increase in the distance between peaks but considerably prolonged the separation time. Normal butyl ether alone eluted the mono acids from C_6 to C_{16} in one peak, but separated the mono acids from C_2 to C_5. It was also found that *n*-butyl ether, alone or in mixtures with Skellysolve B, was an excellent solvent for dicarboxylic acids.

ACKNOWLEDGMENT

The author is indebted to W. D. Lewis of the Wisconsin Alumni Research Foundation for valuable suggestions and assistance in writing the report.

The author also wishes to express his gratitude to Henry A. Schuette, University of Wisconsin, V. P. Kuceski, C. P. Hall Co., Chicago, Ill., and to R. N. Meyer, Standard Oil Research Laboratory, Chicago, Ill., for gracious gifts of samples of some of the higher molecular weight acids.

LITERATURE CITED

(1) Begemann, P. H., Keppler, J. G., and Boekenoogen, H. A., *Rec. trav. chim.*, **69**, 439 (1950).
(2) Boelhouwer, C., Gerckens, J., Lie, O. T., and Waterman, H. I., *J. Am. Oil Chemists' Soc.*, **30**, 59 (1953).
(3) Higuchi, T., Hill, N. C., and Corcoran, G. B., ANAL. CHEM., **24**, 491 (1952).
(4) Isherwood, F. A., *Biochem. J.* (*London*), **40**, 688 (1946).
(5) Nijkamp, H. J., *Anal. Chim. Acta*, **5**, 325 (1951).
(6) *Ibid.*, **10**, 448 (1954).
(7) Ramsey, L. L., and Patterson, W. I., *J. Assoc. Offic. Agr. Chemists*, **28**, 744 (1945).
(8) *Ibid.*, **31**, 139, 480 (1948).
(9) Vandenheuvel, F. A., and Hayes, E. R., ANAL. CHEM., **24**, 960 (1952).
(10) Zbinovsky, V., and Burris, R. H., *Ibid.*, **26**, 209 (1954).

RECEIVED for review October 26, 1954. Accepted January 18, 1955. Study carried out in the laboratories of the Wisconsin Alumni Research Foundation and supported by a grant from Consolidated Badger Co-operative, Inc., Shawano, Wis.

Isonicotinic Acid Hydrazide as a Reagent for Determination of Δ^4-3-Ketosteroids

Determination of Progesterone and Testosterone Propionate in Oil Solutions

ERNEST J. UMBERGER

Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D. C.

A new color reaction suitable for quantitative determination of Δ^4-3-ketosteroids and based upon the rapid formation of their hydrazones from isonicotinic acid hydrazide in absolute alcohol solutions acidified with hydrochloric acid is described. A yellow color having an absorption maximum at 380 mμ is developed. The method has been applied to the analysis of vegetable oil solutions of testosterone propionate and progesterone. When the concentration of steroid in the oil is 10 mg. per ml. or less, separation of the steroid from the oil may be necessary. A chromatographic technique for this purpose is described. Methods are described for the quantitative separation of testosterone propionate and progesterone by chromatography and the identification of testosterone propionate in vegetable oil solutions.

ISONICOTINYL hydrazones of several ketosteroids have been prepared by Ercoli, de Giuseppe, and de Ruggieri (*3*). These authors observed that when the isonicotinyl hydrazones of a ketosteroid having a double bond conjugated with a carbonyl group were dissolved in alcohol, and weakly acidified with acetic acid, a clear yellow color was produced. Hydrazones of saturated ketosteroids, or those having the double bond in other than the alpha, beta-position, did not give a color.

If hydrochloric acid is substituted for acetic acid, a more intense yellow color is obtained. Furthermore, the rate of formation of the hydrazones from Δ^4-3-ketosteroids at room temperature is rapid. Based on these observations, a method for the colorimetric estimation of Δ^4-3-ketosteroids has been developed and applied to the determination of progesterone and testosterone propionate in vegetable oil solutions.

REAGENTS AND APPARATUS

Isonicotinic Acid Hydrazide Reagent. Isonicotinic acid hydrazide (melting point, 171.5° to 172.5° C.), 500 mg., was dissolved in absolute ethanol. Concentrated hydrochloric acid, 0.625 ml., was added and the solution was made up to 1 liter with absolute ethanol.

Standard Solutions. Stock solutions of United States Pharmacopeia reference standards of progesterone and testosterone propionate containing 100 γ per ml. were prepared in absolute ethanol.

All other reagents and solvents were reagent grade and were used without further purification.

Reaction Vessels. Cylindrical centrifuge tubes approximately 25 × 150 mm. fitted with 24/25 standard-taper ground-glass stoppers were used. These could be readily attached to a reduced pressure still head fitted with a 24/40 standard-taper joint and a capillary tube for distillation of aliquots of the samples to dryness.

EXPERIMENTAL

The isonicotinic acid hydrazide reagent described was the result of a careful study of the influence of several variables on the color formation and the quantitative applications of the reaction. While most of the experiments were performed using testosterone propionate and progesterone, sufficient experiments were made with other ketosteroids to show that the conclusions reached applied equally well to them. The basic procedure was as follows:

An aliquot of an ethanolic solution of the steroid containing 50 γ was measured into a reaction vessel. The solution was distilled to dryness and 4 ml. of the isonicotinic acid reagent under study were added. The tube was stoppered and agitated to dissolve the steroid, and allowed to stand 1 hour at room temperature. The absorbance was measured on the Beckman Model B spectrophotometer using a 1.0-cm. Corex cell at 380 mμ, against a corresponding reagent blank.

Choice of Solvent. Only absolute methanol or absolute ethanol has been found satisfactory as a solvent for the reaction. Other solvents were unsatisfactory because of the limited solubility of the hydrochloride of isonicotinic acid hydrazide in them. Those studied were 1-propanol, isopropanol, 1-butanol, chloroform, benzene, acetone, ethyl ether, and ethyl acetate.

Increasing the concentration of acid to 1.0 ml. of concentrated hydrochloric acid per liter also caused precipitation from absolute ethanol on standing. On the other hand, absolute methanol solutions of 10 to 15 times as much hydrazide and acid were stable. Furthermore, the intensity of the color developed under equivalent conditions was about 10% greater in methanol than in ethanol. However, the vegetable oils, which are the usual solvents for progesterone and testosterone propionate in drug preparations, are much less soluble in methanol than in ethanol. Therefore, absolute ethanol appeared to be the choice of solvent for these determinations.

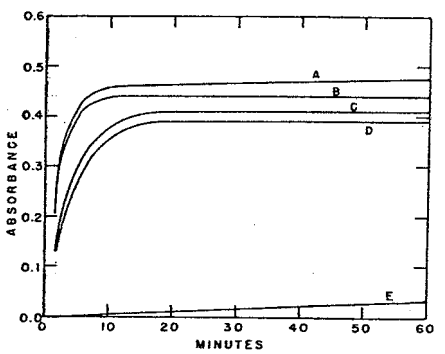


Figure 1. Rate of reaction at 380 mμ in methanol and in ethanol

Steroid concentration, 50 γ/4 ml.
A. Progesterone in methanol reagent
B. Testosterone propionate in methanol reagent
C. Progesterone in ethanol reagent
D. Testosterone propionate in ethanol reagent
E. 7-Ketocholesterol in either methanol or ethanol reagents

Rate of Reaction. For this study identical reagents were prepared in absolute methanol and in absolute ethanol by dissolving 500 mg. of isonicotinic acid hydrazide and 0.625 ml. of concentrated hydrochloric acid in 1 liter of solvent. Fifty micrograms of the dry steroid were dissolved in 4 ml. of the reagent at a room temperature of 30° to 31° C., transferred to the cells of the Beckman spectrophotometer, and the absorbance at 380 mμ was measured at regular intervals from 1.5 to 60 minutes after mixing. The curves so obtained are shown in Figure 1. In ethanol, the reaction appeared to be complete in 20 minutes for both progesterone and testosterone propionate, whereas in methanol the reaction was virtually complete in 10 to 12 minutes. To test the effect of reagent concentration on the rate of reaction, a reagent was prepared having 8 times as much hydrazide and acid in methanol. One-half milliliter of this was added to 50 γ of testosterone propionate, and later diluted with 3.5 ml. of methanol for reading. Under these conditions, the reaction was found complete in less than 2 minutes.

It was of interest to see whether ketosteroids having the alpha, beta-unsaturated ketone grouping in other than ring A would behave similarly in the reaction. For this study, 7-ketocholesterol acetate which has the grouping in ring B was chosen. Curve E, Figure 1, shows the rate at which the absorbance increased with 50 γ of this compound. In comparison, the absorbance was about 10% of what might be expected on a molecular weight basis.

From Figure 1, it can be deduced that readings taken any time after 20 minutes would be satisfactory for either the methanol or ethanol reagent. For practical purposes, 1 hour was selected as the most convenient time to allow the reaction to proceed.

Kind of Acid and Acid Concentration. Keeping the isonicotinic acid hydrazide concentration at 0.5 mg. per ml., and varying the concentration of hydrochloric acid, curve *A*, Figure 2, was obtained using methanol as the solvent. This curve shows that the maximum absorbance was obtained when the concentration of concentrated hydrochloric acid was 0.625 ml. per liter. This concentration of acid was found to be 0.0074*N* (or molar) when titrated against standard alkali. Since a concentration of 0.5 mg. per ml. for isonicotinic acid hydrazide is 0.00365*M*, the greatest absorbance occurs when the molar ratio of acid to hydrazide is two to one. Similar results were obtained in ethanol, and with 0.0074*N* sulfuric acid. On the other hand, the concentration of acetic acid had to be increased to about 25% by volume (approximately 4.4*N*) before any appreciable effect occurred.

Table I. Melting Points of Isonicotinyl Hydrazones of Some Ketosteroids

Ketosteroid	Melting Point, ° C.
Androsterone	276–7
Testosterone	216–18[a]
Testosterone propionate	192–4
Methyl testosterone	216–18
Progesterone	Soft. 167, m. 178–80
Cortisone	Soft. 183, m. 187–9
Hydrocortisone	Soft. 173, m. 180–2

[a] Literature value (*3*) is 218° to 220° C.

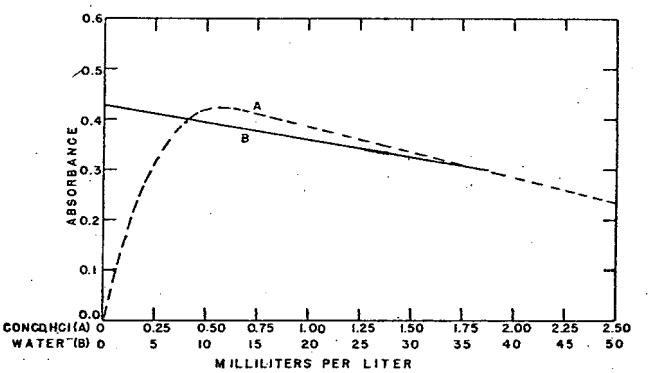


Figure 2. Effect of concentration on absorbance at 380 mμ from 50 γ of testosterone propionate in methanol reagent

A. Concentrated hydrochloric acid
B. Water

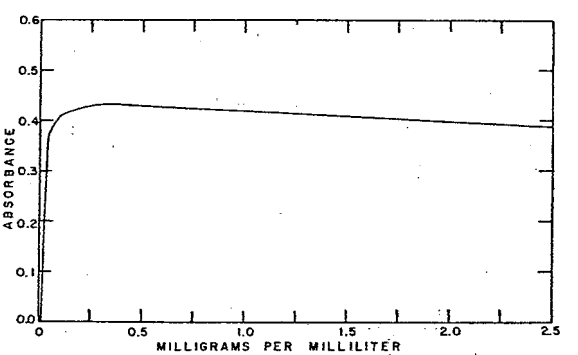


Figure 3. Effect of concentration of isonicotinic acid hydrazide on absorbance at 380 mμ from 50 γ of testosterone propionate in methanol reagent

Effect of Water Concentration. Curve *B*, Figure 2, shows the effect of varying the concentration of water when methanol was the solvent. Here the isonicotinic acid hydrazide and the acid concentration were kept constant at 0.5 mg. per ml., and 0.625 ml. per liter, respectively.

Isonicotinic Acid Hydrazide Concentration. Figure 3 shows that the greatest absorbance is obtained when the isonicotinic acid hydrazide concentration is between 0.25 and 0.5 mg. per ml.

The hydrochloric acid concentration was kept at a constant molar ratio of two to one with respect to isonicotinic acid hydrazide.

Effect of Temperature, Light, and Oxygen. Varying the temperature from 10° to 100° C. markedly affected the rate at which the absorbance increased but did not materially affect the final absorbance at 380 mμ. The reaction was not affected by light. While the reaction was generally carried out in a stoppered tube to prevent evaporation of the solvent or the absorption of moisture from the air, there was no indication that the presence or absence of oxygen affected the reaction in any way.

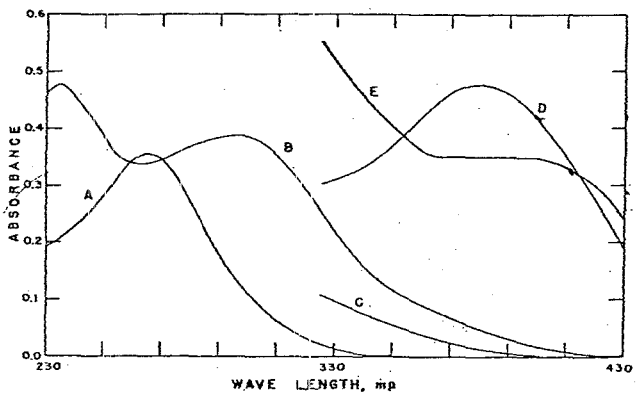


Figure 4. Absorption spectra of isonicotinyl hydrazones

A. Androsterone, 14.2 γ/ml., in absolute ethanol
B. Testosterone, 10.0 γ/ml., in absolute ethanol
C. Androsterone, 14.3 γ/ml., in ethanol reagent
D. Testosterone, 17.7 γ/ml., in ethanol reagent
E. Reaction mixture of benzal acetone, 5.0 γ/ml., in ethanol reagent

Preparation of Isonicotinyl Hydrazones. In order to develop data bearing on the chemistry of the color reaction, the hydrazones of several ketosteroids were prepared, and their uncorrected melting points are shown in Table I. The hydrazones of the first four ketosteroids listed were easily prepared by the following procedure:

Thirty milligrams of isonicotinic acid hydrazide and 60 to 75 mg. of the steroid (molar ratio roughly 1.1 to 1.0) were dissolved in 5 ml. of absolute alcohol and 0.25 ml. of alcoholic hydrochloric acid (prepared by diluting 2.5 ml. of concentrated hydrochloric acid to 100 ml. with absolute alcohol). The mixture was heated on the steam bath 0.5 hour and transferred to a separatory funnel with 15 ml. of chloroform and 25 ml. of water. After shaking, the chloroform layer was separated and the aqueous layer extracted two times with 10-ml. portions of chloroform. The combined chloroform extracts were washed three times with 10-ml. portions of water and evaporated to dryness. The hydrazones were recrystallized from 50% ethanol or methanol to a constant melting point.

The hydrazones of progesterone, cortisone, and hydrocortisone were found more difficult to prepare and purify. These steroids have two or more carbonyl groups of varying reactivity, and the reaction products, therefore, were likely complicated mixtures. Their purification was further complicated by the fact that the reaction products from cortisone and hydrocortisone were water-soluble, making it difficult to separate the hydrazones from unreacted isonicotinic acid hydrazide. Their preparation was finally accomplished by the following general procedure:

Fifty milligrams of the steroid were mixed with sufficient isonicotinic acid hydrazide to make a molar ratio of steroid to hydrazide of 1 to 5, and the mixture dissolved in sufficient 0.3*N* hydrochloric acid in absolute methanol to make a molar ratio of hydrazide to acid of 1 to 3. In the cases of progesterone and cortisone, the solution was heated on the steam bath for 1 hour. The hydrocortisone preparation was allowed to stand at room temperature for 0.5 hour. The mixtures were diluted with 10 to 15 ml. of water and neutralized with 0.1*N* sodium hydroxide. The hydrazone of progesterone was filtered off, and those of cortisone and hydrocortisone extracted immediately with chloroform from the aqueous solution. After evaporation of the chloroform, the residues were recrystallized first from a mixture of chloroform and ether and finally from chloroform. Sharp melting points for these hydrazones were not obtained. The crystals would appear to soften and later change to a plastic mass with darkening and with liquefaction. This was recorded as the melting point in Table I.

The formation of hydrazones from isonicotinic acid hydrazide and Δ^4-3-ketosteroids is a reversible reaction. The yellow color of an acid-alcohol solution of a Δ^4-3-ketosteroid hydrazone will disappear within a few hours. However, in the presence of an excess of isonicotinic acid hydrazide, the color is stable for a week or more in a stoppered flask. An ethanolic solution of the testosterone hydrazone was diluted with water and made strongly acid with hydrochloric acid. On standing, crystals of testosterone were deposited which were identified by their melting point. This ease of hydrolysis of isonicotinyl hydrazones is in marked contrast to that found by others (*1, 2, 5*) who found other hydrazones of Δ^4-3-ketosteroids very difficult to cleave.

Absorption Spectra. The absorption spectra in the region 230 to 430 mμ of two typical ketosteroid hydrazones are shown in Figure 4. A Beckman Model DU spectrophotometer with 1.0-cm. quartz cells was used for these determinations. Curve *A* shows the spectrum of the hydrazone of androsterone, a saturated ketosteroid, and curve *B* shows the spectrum of the hydrazone of testosterone, a Δ^4-3-ketosteroid, in absolute alcohol. These spectra are similar to those given by Ercoli and coworkers (*3*) for the isonicotinyl hydrazones of cholestanone and cholestenone,

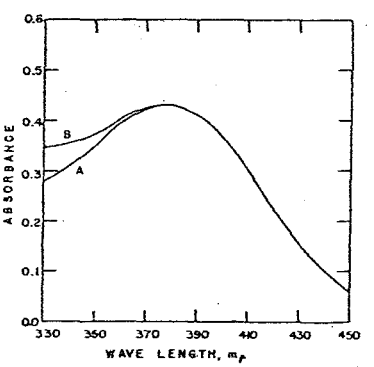


Figure 5. Comparison of absorption spectra

Progesterone, 50 γ/4 ml., in ethanol reagent
A. After 20 minutes
B. After 18 hours; also, hydrazone of progesterone, 87.9 γ/4 ml., immediately after dissolving

respectively. These authors state that the ultraviolet absorption spectra are not changed by acidification of the alcoholic solutions of these hydrazones with acetic acid. A similar situation was found with respect to testosterone isonicotinyl hydrazone, provided the concentration of acetic acid remained below 25% by volume. In 50% acetic acid and in glacial acetic acid, a new maximum appeared at about 360 mμ. With hydrochloric and sulfuric acids, however, this new maximum occurred at 380 mμ at very dilute concentrations of acid. Curve *D*, Figure 4, shows the effect of hydrochloric acid on testosterone isonicotinyl hydrazone, whereas curve *C* shows that hydrochloric acid has little effect on androsterone isonicotinyl hydrazone. Because hydrazones are rapidly hydrolyzed in acid solution in the absence of an excess of isonicotinic acid hydrazide, it was not possible to obtain accurate absorption spectra of these hydrazones in the presence of acid only. Curves *C* and *D*, therefore, show the absorption of the hydrazones of androsterone and testosterone, respectively, in the presence of 0.0074*N* hydrochloric acid and 0.5 mg. per ml. of isonicotinic acid hydrazide, compensated for

by a suitable blank. The presence of the hydrazide renders the solution opaque below 325 mμ.

Absorption spectra following the treatment of various steroids with the isonicotinic acid hydrazide reagent under the conditions of the reaction procedure were obtained also. Treatment of 100 γ of androsterone with 4 ml. of the ethanol reagent for 1 hour at room temperature resulted in no measurable absorption between 325 and 430 mμ. This same solution allowed to stand at room temperature for 24 hours exhibited an absorption spectrum similar to curve *C*, Figure 4.

A solution of testosterone isonicotinyl hydrazone was prepared in the ethanol reagent and its spectrum, determined immediately, compared with the spectrum of an equimolecular amount of testosterone carried through the 1-hour reaction procedure. The curves were identical and there was no change on standing. This experiment demonstrates the facts that the end product in the color reaction is an isonicotinyl hydrazone of the Δ^4-3-keto group and that the reaction is quantitative.

A similar experiment with progesterone gave slightly different curves. Curve *A*, Figure 5, is the spectrum of the reaction mixture 20 minutes after adding the ethanol reagent to 50 γ of progesterone. Curve *B* shows the spectrum of the same solution 18 hours later. When an equimolecular amount of the hydrazone of progesterone (87.9 γ) was dissolved in the same amount of ethanol reagent and read immediately, a spectrum identical to curve *B* was obtained.

Chemistry and Specificity of the Reaction. The above experiments suggest the following with respect to the chemistry of the color reaction. When a steroid having a carbonyl group is treated with isonicotinic acid hydrazide in absolute alcohol weakly acidified with hydrocholoric acid, a hydrazone is formed. The Δ^4-3-keto group reacts quantitatively at room temperature in less than 1 hour. Other keto groups react only after long standing or after heating. Dilute hydrochloric acid acts not only as a catalyst in the reaction but profoundly changes the absorption spectrum of the hydrazone formed with the Δ^4-3-keto group so that its acid-alcohol solution exhibits a yellow color and absorbs in the visible region of the spectrum with an absorption maximum at 380 mμ. This change is illustrated by curves *B* and *D*, Figure 4, for the hydrazone of testosterone. Hydrazones of other keto groups do not exhibit this change as illustrated by the 17-keto group of androsterone shown by curves *A* and *C*, Figure 4.

Curve *A*, Figure 5, shows the absorption spectrum of the hydrazone of progesterone when only the Δ^4-3-keto group has reacted and curve *B* shows the spectrum obtained when both the Δ^4-3-keto group and the 20-keto group have reacted. The two curves are different in the region 330 to 380 mμ. If the absorbance of curve *A* is subtracted from that of curve *B* in this region, and this difference plotted against the wave length, a curve similar to curve *C*, Figure 4, is obtained. The 20-keto group of progesterone, therefore, appears to react much more slowly than the Δ^4-3-keto group and its hydrazone gives an absorption spectrum similar to the hydrazone of the 17-keto group of androsterone. Because cortisone and hydrocortisone behaved like progesterone in parallel experiments, it may be concluded that the 20-keto groups of these compounds behave like the 20-keto group of progesterone, and that the 11-keto group of cortisone failed to react.

Since the rate of reaction and the absorption spectra obtained indicated that the reaction was highly specific for Δ^4-3-ketosteroids, the behavior of a variety of compounds was tested:

The compound to be tested was dissolved in absolute ethanol at concentrations up to 100 γ per ml. A double-strength reagent was prepared by dissolving isonicotinic acid hydrazide to a concentration of 1.0 mg. per ml. in absolute ethanol acidified with 1.25 ml. of concentrated hydrochloric acid per liter. Two milliliters of the solution of the compound under test was mixed with 2 ml. of the reagent and the mixture was allowed to stand at room temperature for 1 hour. The absorption spectra were determined.

Table II. Molecular Weights of Hydrazones

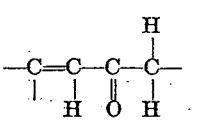
Ketosteroid	Mol. Wt. of Derivative Calcd. as Mono-substituted	Di-substituted	Tri-substituted	Mol. Wt., Found
Testosterone	407.53	...	...	406.90
Testosterone propionate	463.60	...	...	463.74
Methyl testosterone	421.56	...	...	430.81
Progesterone	433.57	552.69	...	555.25
Cortisone	479.55	598.67	717.69	606.79
Hydrocortisone	481.57	600.69	...	600.65

Compounds which gave an absorption spectrum similar to curve *D*, Figure 1, and exhibited a yellow color were: progesterone, testosterone, testosterone propionate, methyl testosterone, cortisone, cortisone acetate, hydrocortisone, desoxycorticosterone acetate, and 7-ketocholesterol acetate. The reaction with 7-ketocholesterol acetate was incomplete in 1 hour. With the exception of 7-ketocholesterol acetate, all of these compounds were steroids having the alpha, beta-unsaturated keto group in ring A.

Two nonsteroidal ketones which gave a yellow color in the reaction were benzal acetone and benzyl styryl ketone. The former reacted much more rapidly than the latter. Their absorption spectra were nearly identical but differed from those given by the steroids. For comparison, the absorption spectrum for the isonicotinyl hydrazone of benzal acetone formed in the reaction is shown as curve *E*, Figure 4.

Compounds tested which gave no color in the reaction were androsterone, estrone, benzaldehyde, phenyl acetone, benzal acetophenone, acetone, ethyl aceto acetate, and ascorbic acid. Androsterone and estrone gave absorption in the region studied only after long standing. Benzaldehyde, phenyl acetone, and benzal acetophenone gave reaction mixtures with absorption spectra resembling curve *C*, Figure 4, after 1 hour, but no attempt was made to determine how much of this absorption may have been due to the parent compound. Absorption spectra for the other reaction mixtures were not obtained.

Although the number of compounds tested is limited, it would appear the rapid formation of a hydrazone having an absorption spectrum with a maximum at 380 mμ and exhibiting a yellow color is dependent upon the presence of the grouping



in the molecule. Any Δ^4-3-ketosteroid, therefore, would react. Other steroids having this grouping in other positions were not available for testing. Of course, interference would be expected from any compound or substance which shows absorption at 380 mμ.

Since practically all of the absorbance at 380 mμ in the reaction is contributed by the hydrazone of the Δ^4-3-keto group, the molecular weight of the hydrazone derivative can be estimated by measuring the amount of the derivative dissolved in the reagent necessary to give an absorbance equal to that of a known amount of the original steroid in the reaction mixture. In this manner the molecular weights of the various hydrazones prepared were estimated and the results are given in Table II, which shows that the derivative isolated in the preparation of the hydrazones from progesterone, cortisone, and hydrocortisone was in every case the di-substituted derivative.

Sensitivity. In order to compare the sensitivity of this method with other colorimetric methods for steroids, molar extinction coefficients ($\epsilon = A/lxc$, where A is absorbance, l is light path in centimeters, and c is concentration in moles per liter) were calculated. These were found to average about 11,000 for the ethanol reagent and about 12,000 for the methanol rea-

gent. These compare with coefficients of about 17,000 for the ultraviolet absorption method at 240 mμ and about 20,000 to 25,000 by other colorimetric methods (*4*, *6*, *8*). Gornall and MacDonald (*4*) found a 2,4-dinitrophenyl-hydrazine reagent extremely sensitive for cortisone and hydrocortisone, having obtained molar extinction coefficients of about 45,000 for these two compounds when more than one carbonyl group was forced to react.

Table III. Analysis of Known Oil Solutions of Testosterone Propionate and of Progesterone

Concn., Mg./Ml.	Found: Testosterone propionate Mg./ml.	%	Progesterone Mg./ml.	%
10.0	10.4	104.0	10.4	104.0
25.0	24.9	99.6	25.0	100.0
	25.0	100.0	25.1	100.4

Table IV. Analysis of Progesterone in Various Oils

Sample No.	Concn., Mg./Ml.	Kind of Oil	Before Chromatography Mg./ml.	%	After Chromatography Mg./ml.	%
1	2	Peanut	3.24	162	2.04	102
2	2	Sesame (*A*)	2.86	143	2.00	100
3	25	Sesame (*A*)	26.5	106	23.5	94
4	25	Sesame (*B*)	25.0	100	25.6	103
5	25	Sesame (*B*) with 3% benzyl alcohol	25.0	100	25.0	100

Chromatographic Separation of Testosterone Propionate and Progesterone from Vegetable Oil Solutions. When the concentration of testosterone propionate or progesterone in vegetable oils was 10 mg. per ml. or less, materials in the oil contributed significantly to the absorption at 380 mμ. Peanut oil gave more absorption than corn oil, and corn oil more than sesame oil. The absorption was greater in oils which had been exposed to the air for some time. The error could be completely compensated for by dissolving an equivalent amount of the same batch of oil in the standard solution as an internal blank. Except in manufacturing plants, a sample of the same oil generally is not available. An attempt was made, therefore, to remove the substances responsible for the increased absorption by chromatographic techniques. During this investigation, conditions were discovered for quantitative separation of testosterone propionate and progesterone by chromatography. The column used was that described by Umberger and Curtis (*10*).

Florisil (60- to 100-mesh), 4.25 grams, was slurried into the column with a solvent mixture of two parts of iso-octane and one part of chloroform. A solution containing 1.25 mg. of testosterone propionate and 1.0 mg. of progesterone in 5 ml. of the same solvent mixture was added, and washed in with two more 5-ml. portions. The column was then developed with 100 ml. of the same solvent mixture. The testosterone propionate was eluted with 200 ml. of a solvent mixture of one part of iso-octane and one part of chloroform, following which the progesterone was eluted with 150 ml. of chloroform. Recoveries were quantitative.

Experiments in which various oils were chromatographed showed that most of the material which interfered in the color reaction was eluted during the first 100 ml. of developing solvent, some remained on the column at the end of the experiment, and only traces appeared in the testosterone propionate and progesterone fractions.

RECOMMENDED ASSAY PROCEDURE

Using a carefully calibrated tuberculin syringe, a sample of the vegetable oil solution of the steroid is measured out and dissolved in absolute ethanol (not over 1.0 ml. of oil per 100 ml. of solution). An aliquot of this solution containing 50 γ of the steroid is added to a reaction vessel and the ethanol removed by distillation. An aliquot of the standard solution is treated similarly. Four milliliters of the isonicotinic acid hydrazide reagent are added and the stoppered vessel is shaken or rotated to ensure complete dissolution of the sample. The vessel is allowed to stand for 1 hour at room temperature, and the absorbance is measured at 380 mμ against a reagent blank on the Beckman Model B or Model DU spectrophotometer using 1.0-cm. Corex cells.

If the oil contains steroid in the amount of 10 mg. or less, and a sample of the same oil is not available for an internal blank, the steroid may be removed from the oil by chromatographic adsorption. A quantity of the oil solution of the steroid is measured out and dissolved in a solvent mixture of two parts of iso-octane and one part of chloroform, so that each 5 ml. will contain 0.5 mg. of the steroid being determined. A column is prepared with a slurry of 4.25 grams of Florisil (60- to 100-mesh) in the same solvent, and 5 ml. of the sample solution are added. The sample is washed in with two 5-ml. portions of the same solvent mixture, and the column is developed with an additional 100 ml. The testosterone propionate is now eluted with 200 ml. of a solvent mixture of one part of iso-octane and one part of chloroform. This solution is distilled to dryness, the residue is dissolved in absolute ethanol, and an aliquot containing 50 γ is distilled to dryness in a reaction vessel for the assay. The procedure is the same for progesterone, except that the 200 ml. of one to one solvent is discarded, and the progesterone is eluted with an additional 150 ml. of chloroform.

APPLICATION AND RESULTS

Analysis of Progesterone and Testosterone Propionate in Vegetable Oil Solutions. Table III shows the results obtained by the recommended procedure on known sesame oil solutions of testosterone propionate and of progesterone without the use of chromatographic separation of the steroids from their oil solvents. With this particular sample of oil, the results for both progesterone and testosterone propionate were about 4% high at the 10-mg. level. Table IV shows the results of the analysis of some known oil solutions of progesterone in one sample of peanut oil and two samples (*A* and *B*) of sesame oil before and after chromatographic adsorption. Benzyl alcohol, often present in vegetable oil injections, was added to one sample of the sesame oil (*B*) to a concentration of 3%, and did not appear to interfere.

Table V. Comparison of Methods for Analysis of Commercial Preparations of Progesterone in Oil

Sample No.	Labeled Concn., Mg./Ml.	Found: Present method[a] Mg./ml.	%	U.S.P. XIV[b] Mg./ml.	%
1	10	9.4	94.0	9.4	94.0
		9.6	96.0		
2	25	17.0	68.0	16.5	66.0
		17.5	70.0		
3	25	22.3	89.2	22.4	89.6
		22.8	91.2		

[a] Without chromatographic separation.
[b] Modified by use of equilibrated solvents (*9*).

Table VI. Comparison of Analysis of Commercial Preparations of Testosterone Propionate in Oil

Sample No.	Labeled Concn., Mg./Ml.	Found, % of Labeled Claims: Present Method Before chrom.	After chrom.	Semicarbazide method	U.S.P. XIV method
1	5	122	106	109	...
		127	107		
2	10	106	96	117	91 ± 12
		111	95		
3	25	92	...	101	88 ± 8
		94			
4	25	84	...	76	119 ± 10
		86			
5	25	102	95	102	106 ± 9
		100	98		
6	25	106	...	101	110 ± 16
		108			
7	25	100	...	94	95 ± 15
		101			
8	50	87	...	84	102 ± 8
		86			
9	50	98	...	...	110 ± 10
		99			
10	50	97	98	114	108 ± 15
		100	95		
11	100	102	...	119	96 ± 13
		94			
12	100	97	...	117	...
		97			

Tables V and VI show the results of the analysis of three commercial preparations of progesterone in oil and 12 commercial samples of testosterone propionate in oil without chromatographic separation from the oil. A few of the testosterone propionate samples at each concentration level were analyzed also using the chromatographic technique. For comparison, the progesterone samples were analyzed also by a slight modification of the U. S. Pharmacopeia XIV method for progesterone injection (*9, 11*), and the testosterone propionate samples by the semicarbazide method of Madigan, Zenno, and Pheasant (*7*) and by the bioassay method of the U.S.P. XIV for testosterone propionate injection (*12*). The author experienced great difficulty in filtering the semicarbazones formed in the semicarbazide method, and the results in many of the cases are probably high.

Identification of Testosterone Propionate in Oil. Because the isonicotinyl hydrazone of testosterone propionate could be purified easily, a technique was developed for the identification of testosterone propionate in oil solutions. A quantity of oil containing 25 mg. of testosterone propionate and 50 mg. of isonicotinic acid hydrazide were added to 5 ml. of an acid-alcohol mixture containing 1.25 ml. of concentrated hydrochloric acid per 100 ml. of absolute alcohol. The mixture was refluxed on the steam bath for 15 minutes. After cooling, the mixture was diluted with 25 ml. of ether. The precipitated hydrochlorides of isonicotinic acid hydrazide and the hydrazone of testosterone propionate were collected on a coarse sintered-glass filter and washed with 15 ml. of ether. The residue was transferred to a separatory funnel with 10 ml. of water and 10 ml. of chloroform. After shaking and separating the layers, the aqueous layer was washed with 5 ml. of chloroform. The combined chloroform solutions were washed three times with 5-ml. portions of water, filtered, and evaporated to dryness. The residue was recrystallized once from 50% ethanol. After drying, the hydrazone melted at 192° to 4° C.

LITERATURE CITED

(1) Anchel, M., and Schoenheimer, R., *J. Biol. Chem.*, **114**, 539–46 (1936).
(2) Djerassi, C., ANAL. CHEM., **20**, 880–1 (1948).
(3) Ercoli, A., de Giuseppe, L., and de Ruggieri, P., *Farm. sci. e tec. (Pavia)*, **7**, 170–2 (1952).
(4) Gornall, A. G., and MacDonald, M. P., *J. Biol. Chem.*, **201**, 279–97 (1953).
(5) Klein, D., Weiner, N., and Gordon, S. M., ANAL. CHEM., **20**, 174–6 (1948).
(6) Mader, W. J., and Buck, R. R., *Ibid.*, **24**, 666–7 (1952).
(7) Madigan, J. J., Zenno, E. E., and Pheasant, R., *Ibid.*, **23**, 1691–2 (1951).
(8) Porter, C. C., and Silber, R. H., *J. Biol. Chem.*, **185**, 201–7 (1950).
(9) Umberger, E. J., *J. Am. Pharm. Assoc., Sci. Ed.*, **39**, 700 (1950).
(10) Umberger, E. J., and Curtis, J. M., *J. Biol. Chem.*, **178**, 269 (1949).
(11) "United States Pharmacopeia XIV," p. 493, Mack Publishing Co., Easton, Pa., 1950.
(12) *Ibid.*, p. 609.

RECEIVED for review February 13, 1954. Accepted January 10, 1955.

Determination of Carbon in Sodium-Potassium Alloy

K. G. STOFFER and J. H. PHILLIPS
The Babcock & Wilcox Co., Research Center, Alliance, Ohio

Carbon contained in sodium-potassium alloy, one of the heat exchange media being considered for use in nuclear reactors, can seriously weaken stainless steel container material by carburizing the steel to an appreciable depth. The method described was developed for determining the carbon content of sodium-potassium alloy being used in heat transfer studies. The method developed for this purpose is a modification of the microcombustion procedure and involves the ignition of a 100-mg. sample to convert any carbon present to carbon dioxide which can then be absorbed and weighed. Analytical results are accurate to within tolerances acceptable in microcombustion analysis. The method described also covers the problem of sampling hot sodium potassium alloy from dynamic systems, and the transfer of the sample from the sampling site to the specially designed analytical equipment.

CAREFULLY controlled tests have demonstrated that the 25-20 and 18-8 type alloys can be quickly carburized to an appreciable depth when in contact with sodium-potassium alloy to which carbon has been added. Carburization of the alloys of construction weakens these materials, especially under impact loads. Therefore, it became necessary to develop a method for determining the carbon content of sodium-potassium alloys (hereafter referred to in the text as NaK) so that the carbon content could be ascertained before the liquid alloy was used for filling a given system.

A literature survey revealed little positive information regarding analytical procedures for carbon impurities in the alkali metals. However, in considering the classical method of carbon determination by microcombustion, it was felt that the most promising approach to the NaK carbon problem lay in some modification of the method which has been in use since the time of Pregl (*6*).

It was obvious from the start that considerable revision of the conventional micromethod would be necessary before it could be adapted to NaK analysis. The very nature of this reactive material required completely different methods of sampling, weighing, and introducing of the sample into the combustion tube.

The sampling and analytical methods described below have been proved to be quite satisfactory. Total time required for an analysis is comparable with that for regular carbon microanalysis, but the number of details needing attention during an analysis is considerably larger. The combustion tube in particular, and the attendant apparatus in general, gets more severe usage than do conventional combustion trains. The result is that more than the usual amount of maintenance time is required.

ANALYTICAL APPARATUS

The analytical apparatus will be considered in three parts: the gas purification systems, the sample introduction apparatus, and the combustion tube. A diagram and over-all photograph of the completely assembled combustion train are shown in Figures 1 and 2.

Gas Purification Systems. To obtain oxygen of sufficient purity, cylinder oxygen, at a regulated low pressure, is passed through an absorption train of Ascarite, Drierite, phosphorus pentoxide, and hot copper oxide, followed by a micro bubble counter and Ascarite-Drierite U-tube. The capacity of the train is designed to effect purification of the oxygen flowing at a rate in excess of the 20 cc. per minute which are required at the initial moment of NaK ignition. The purified oxygen enters the combustion tube via side arm *O* (Figure 1).

Chemically pure nitrogen is obtained by passing cylinder nitrogen of prepurified grade through a pressure reducing valve, from

which it passes through a tube of hot degreased copper turnings followed by an absorption train similar to that used for removing impurities from the oxygen stream. The purified nitrogen enters the sample introduction apparatus portion of the combustion train via side arm *N* (Figure 1). Constant pressure in the nitrogen purification system is safeguarded by a mercury bubbler-type release tube.

A separate air purification system is used to provide air for flushing the oxygen out of the absorption tubes before each weighing. Compressed air supplied from a bench outlet is passed through a trap to remove oil, and then through ascarite and drierite of sufficient amount to remove all carbon dioxide and water. Pressure in the air purification system is regulated by a standard micro pressure regulator.

Combustion Tube and Sample Introduction Chamber. Generally, in microcombustion work, introducing the sample into the combustion tube presents no problem. This phase of NaK analysis, however, required the use of special apparatus and procedures.

The combustion tube (Figure 3) is made of transparent quartz. Its design is similar to that of standard microcombustion tubes except for an enlarged combustion chamber designed to accommodate larger sample boats than are used for conventional microcombustions (*5*).

In order to introduce NaK samples into the combustion chamber without exposure to the atmosphere, a sample introduction chamber (Figure 4) was added to the combustion tube at the enlarged end. This introduction chamber is attached to the combustion tube via a 34/28 joint and consists of a 20-mm.-bore, hollow blown stopcock (Figure 1) sealed to a large borosilicate glass tube. This large stopcock serves to separate the inert (nitrogen) atmosphere of the introduction chamber from the oxygen atmosphere of the combustion chamber. A side arm is provided on each side of the stopcock for introducing nitrogen into the introduction chamber and purified oxygen into the combustion chamber. The end of the tube on the introduction side of the stopcock is provided with a 34/28 joint used for attaching either the cap (Figure 4), or the sampler stopcock (Figure 5).

A "push-pull rod" is used for manipulating sample boats in the combustion train. One end of the rod is flattened into a disk and the other end encloses a section of steel rod. The "push-pull rod" is enclosed in a glass tube housing provided with an 18/8 ball joint by means of which it can be attached to either the cap on the introduction chamber, or the sampler stopcock. The other end of the housing is closed except for a 2-mm. hole. By using a magnet external to the housing, the rod can be moved back and forth for manipulating sample boats in the closed system.

The filling of the main (11-mm. outside diameter) section of the combustion tube (Figure 3) is similar to the universal filling of microcombustion carbon-hydrogen tubes (*2*, *4*, *6*). The platinum gauze roll is omitted, however, and a large loose asbestos plug is placed in the 30-mm. outside diameter tube at the constricted end against the copper oxide filling. The purpose of this plug of asbestos is to ensure exposure of the volatilized sample to a heated surface so that the sample will be thoroughly ignited; otherwise vaporized NaK may be carried into the combustion tube filling which is maintained at a lower temperature level and where thermal decomposition of carbonates would be questionable.

To protect the ignition section of the quartz tube against the fluxing action of hot alkali, a loosely fitting, thin-walled, transparent quartz sleeve, 100 mm. long and open at both ends, is inserted into the combustion chamber against the large plug of asbestos. This protective sleeve is expendable and can readily be replaced as frequently as conditions demand.

The sample and large asbestos plug in the ignition chamber are heated by two air-gas, blast-type Fischer burners to a final temperature of 950° C. or above. The section of the combustion tube filled with copper oxide is maintained at 700° C. in a microtube furnace, and the section filled with lead peroxide is heated by the ordinary type microcombustion mortar heater maintained at 180° C.

Portions of the combustion train adjacent to the blast burners can be satisfactorily protected against the high ignition temperatures by using a thin slab of refractory brick placed on either end of the 100-mm.-long sample ignition area. The refractory next to the tube furnace should be backed by a sheet of asbestos paper. The other refractory is backed by a 1-inch-thick sheet of insulation block to protect the borosilicate glass sample introduction apparatus from the heat of the burners. A cooling air jet directed against the standard taper joint between the introduction apparatus and combustion tube supplements the protection afforded by the refractory brick and insulation block.

The absorption tubes used are the usual straight-type Drierite and Ascarite microabsorption tubes. A Mariotte flask is used for assisting in the control of the flow of combustion products through the packed section of the combustion tube and absorption tubes.

REAGENTS

Oxygen, supplied from a cylinder of compressed oxygen of 99.8% purity.

Nitrogen, prepurified grade (99.99% pure) from a compressed gas cylinder.

Sulfuric acid, concentrated reagent grade.

Sulfuric acid, 10% solution by volume.

Magnesium perchlorate, anhydrous granular.

Ascarite, 8–20 mesh.

Copper oxide, reagent grade, wire form, preignited in an atmosphere of oxygen.

Lead peroxide, Special-Micro grade, 12–20 mesh.

Phosphorus pentoxide, reagent grade.

Copper metal, light, degreased turnings.

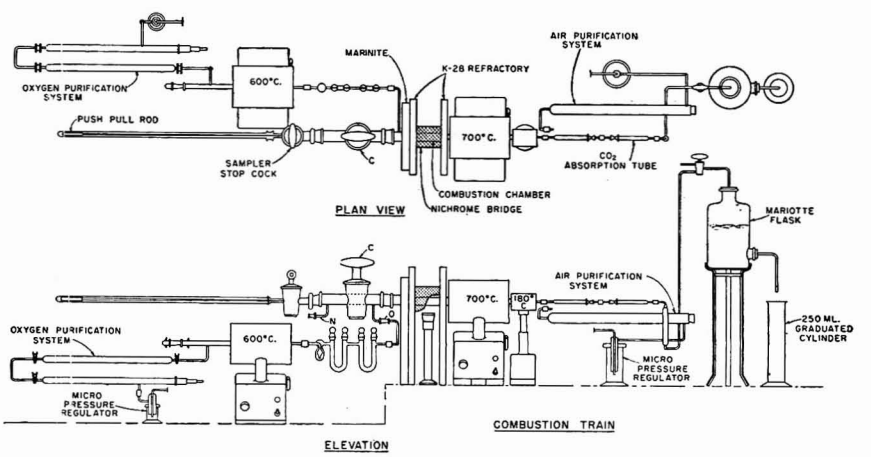


Figure 1. Combustion train

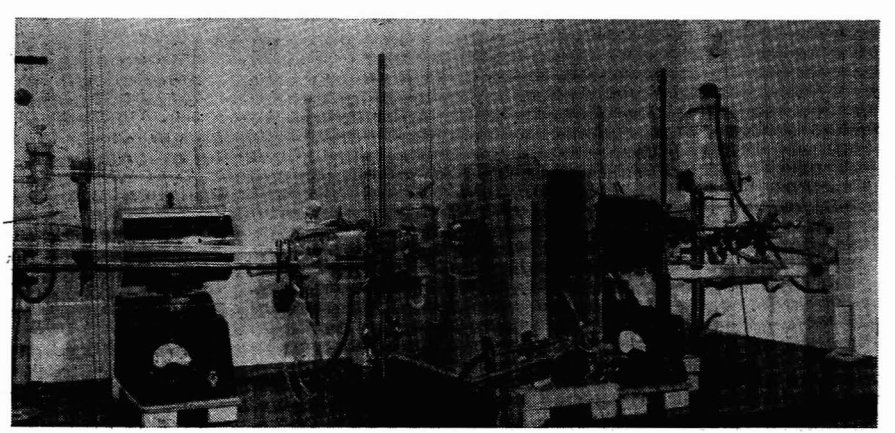


Figure 2. Over-all view of combustion train

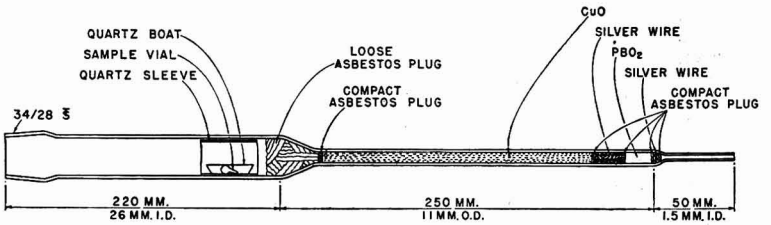


Figure 3. Quartz combustion tube

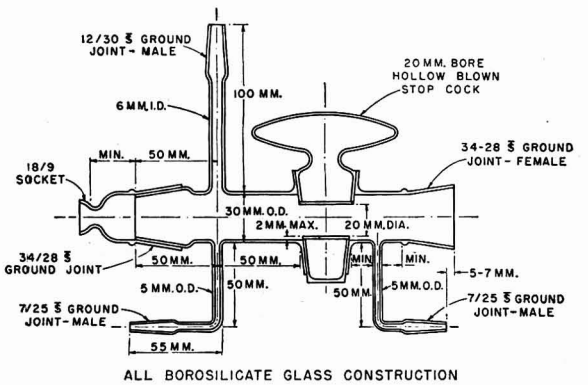


Figure 4. Introduction chamber

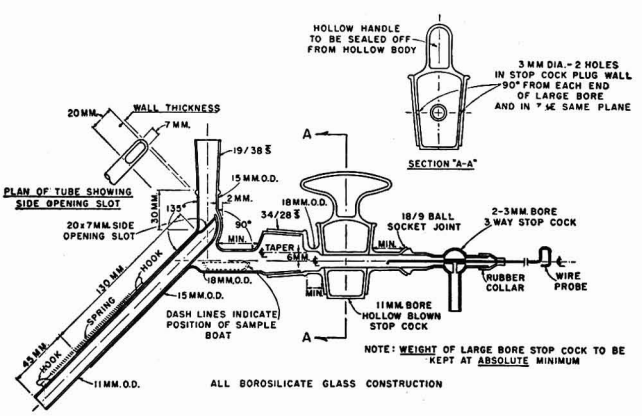


Figure 5. Sampling apparatus

SAMPLING FROM DYNAMIC SYSTEMS

Sampling NaK from a hot dynamic system requires a special technique and the use of specially designed equipment. To be analytically correct, the entire sample must be used for analysis; therefore it must be taken hot, for upon cooling of the sample segregation and precipitation of suspended and dissolved materials may occur. The apparatus described below makes it possible to take a sample that meets these requirements (Figure 5).

It consists of a large bore, hollow blown stopcock equipped on one side with a socket joint and on the other with a standard taper joint. This stopcock, which is lubricated with DC silicone grease, seals the sample from atmospheric contamination during handling, weighing, and transferring to the analytical apparatus. It is attached to a waste tube assembly which is designed to permit discarding waste or contaminated NaK from the sample valve prior to drawing the sample.

The sample valve (Figure 6) which must be an integral part of the system which is being sampled, is constructed without packing, and the fluid seal is accomplished by means of a steel bellows connecting the valve body to the valve stem. The design of the valve is such that manipulation of the valve subjects the bellows to only a spring action with no torsional effects. The valve, as used, is an alteration of a standard valve (No. 1-314) manufactured by the Fulton Sylphon Co., Knoxville, Tenn. The alterations consisted of (*a*) reaming out the normal inlet and outlet connections to provide a straight through passage, (*b*) adding a threaded connection at the bottom, which contains the valve seat, to provide a means of connecting the sampling apparatus, and (*c*) extending the valve stem to reach the new valve seat location.

A metal (Invar) 19/38 joint (Figure 6) provides a tight connection between the sample valve and the sampling apparatus. A removable, thin-walled, stainless steel delivery tube seats against the open valve seat in such a manner as to direct the sample to the waste tube and sample boat from the sample line.

SAMPLING PROCEDURE

Before taking a sample, the sampler stopcock and a clean ignited sample boat are weighed to the nearest milligram. The apparatus (Figure 7) is assembled at the sampling site and prepurified grade cylinder nitrogen is introduced through the 3-way stopcock to purge all the air from the system. Nitrogen flow is maintained throughout the sampling procedure at a rate of 5 to 7 liters per minute. The nitrogen velocity is more than sufficient to prevent significant counter diffusion of air into the apparatus.

When the apparatus has been purged for 3 to 5 minutes with the sample boat in position under the delivery tube and the waste tube in place above it, the sample valve is carefully opened to permit NaK to drop into the waste tube. Waste NaK, drawn

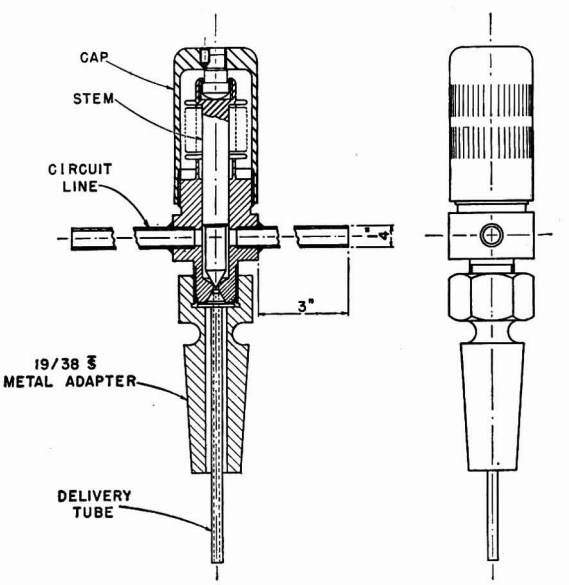


Figure 6. Sample valve assembly

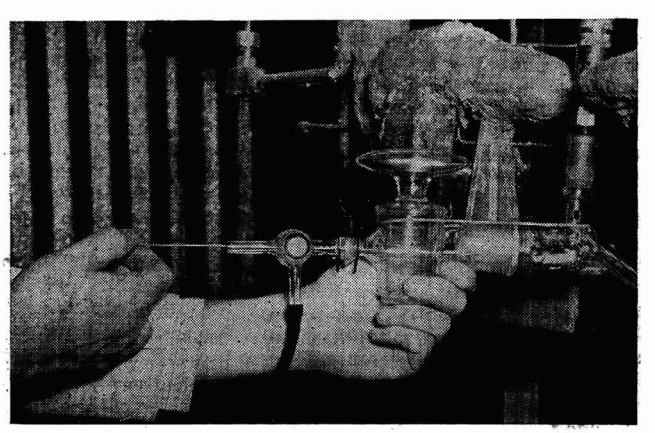


Figure 7. Sampling site

off to cleanse the valve seat of any static alloy, drops into the waste tube and from there into a scoop of potassium chloride crystals and is subsequently discarded. When sufficient waste NaK has been withdrawn, the waste tube is quickly pulled back to permit 2 drops (approximately 100 mg.) of sample to fall into the boat. The waste tube is then returned to its original position, the sample valve is closed, and the boat is pulled into the sampler stopcock, using the wire probe. The stopcock is then closed, sealing the sample within the bore of the stopcock plug which is then removed from the apparatus and reweighed to obtain the sample weight by difference.

TEST PROCEDURE

The train is assembled as shown in Figure 1, with the proper tube fillings as described above. Stopcock *C* is lubricated with silicone grease by applying it in strips 1 cm. in width at the top and bottom of the ground surface.

The standard taper on the combustion tube is twisted firmly into its mate on the introduction apparatus and then held together with steel springs. The rates of flow of the oxygen and of the air used for purging the absorption tubes after combustion of the sample are adjusted to 20 ml. per minute. All furnace temperatures are maintained on a 24-hour basis so that equilibrium conditions will not vary in the different tube fillings.

Table I. Recovery of Carbon Added to NaK Alloy

NaK, Mg.	Additive	Additive, Mg.	Total C Present, Mg.[a]	C Found, Mg.	C Found, %	Deviation, Mg.
103.0	None				0.005	
92.1	None				0.004	
157.5	None				0.004	
98.6	None				0.006	
98.7	None				0.003	
500.0	None				0.005	
600.0	None				0.002	
595.6	None				0.003	
96.0	Na_2CO_3	0.989	0.117	0.123		+0.006
132.3	Na_2CO_3	0.772	0.095	0.099		+0.004
147.7	Na_2CO_3	0.951	0.113	0.110		−0.003
121.7	Na_2CO_3	0.969	0.116	0.109		−0.007
91.2	Na_2CO_3	0.770	0.092	0.086		−0.006
100.5	K_2CO_3	2.150	0.192	0.203		+0.011
116.9	K_2CO_3	1.618	0.149	0.153		+0.004
108.1	K_2CO_3	0.983	0.092	0.097		+0.005
131.7	K_2CO_3	0.640	0.063	0.069		+0.006
103.5	K_2CO_3	1.272	0.116	0.121		+0.005
105.1	Graphite	0.070	0.074	0.068		−0.006
67.1	Graphite	0.217	0.220	0.225		+0.005
140.0	Graphite	0.155	0.161	0.158		−0.003

[a] Includes average of 0.004% carbon found in the NaK used.

Blank determinations follow the test procedure. They are run until they do not exceed 0.030 mg. of carbon dioxide. When satisfactory blank values have been obtained, the combustion train is ready for sample analysis.

COMBUSTION OF SAMPLES

After attaching weighed absorption tubes to the combustion tubes, the sampler stopcock holding the weighed sample is attached to the introduction apparatus. The control rod is then attached to the sampler stopcock and a stream of nitrogen is passed into the introduction chamber to flush all air and carbon dioxide from the entire introduction apparatus, from either side of the sampler stopcock, and from the push-pull rod housing. The sampler stopcock is then opened and the sample boat is pushed into the introduction chamber with the control rod, using a magnet. The large stopcock on the introduction chamber is then opened and the sample is pushed into the quartz sleeve in the combustion chamber. The control rod is then quickly withdrawn and the large stopcock is closed.

The stopcock on the Mariotte flask is then opened and the blast burners are lit. Care must be exercised to maintain oxygen flow in a positive direction at all times. As the temperature of the combustion chamber rises, a close watch is kept for the flash indicating ignition of the sample. At the moment of ignition, the oxygen demand is quite high so that the rate at which it is supplied must be momentarily increased or water will be drawn from the Mariotte flask into the safety tube and absorption tubes.

Completion of the combustion cycle and weighing of the carbon dioxide absorption tube follow closely that of conventional microcombustion analyses.

EXPERIMENTAL RESULTS

To determine the accuracy of the method, a series of tests was conducted on known substances to determine whether all carbon present could be recovered. Weighed quantities of graphite, sodium carbonate, and potassium carbonate were added to ampules of NaK made up following the procedure of Walters and Miller (*7*). The results given in Table I are representative values obtained for each of these substances added to NaK and analyzed as mentioned above.

Results on the above recovery experiments vary from the theoretical amount of carbon added by an average deviation of 0.005% carbon. In conventional microanalysis, deviations of this magnitude are acceptable.

DISCUSSION

When the classical combustion method for carbon analysis was chosen for this investigation, it was realized that high ignition temperatures would be required. While pertinent information on the decomposition temperatures of alkali carbon compounds is lacking, the International Critical Tables refer to two publications (*1*, *3*) each containing small amounts of information. Lebeau gives data on the dissociation pressures of the alkali carbonates at various temperatures. These data indicate that, while dissociation begins at about 700° C., the pressure does not exceed 1 mm. of pressure (mercury) until about 950° C. for sodium carbonate and 1000° C. for potassium carbonate. To obtain this temperature in the manner desired presents a problem, because the combustion chamber must be relatively cool at the time the sample is pushed into place to prevent premature ignition before the system is closed. The use of air-gas blast burners for this purpose proved quite satisfactory. With optimum conditions of line gas pressure, temperatures of 950° C. and higher are obtainable.

With regard to the decomposition of the alkali carbonates, it should be pointed out that the acidic property of the quartz boat itself no doubt assists in the release of carbon dioxide through its reaction with the carbonates. This probability was indicated in recovery experiments; however, poor recoveries of carbon dioxide result unless temperatures of 950° C., or above, are used. This probably is because carbon dioxide remains entrained in the molten mass in the sample boat at the lower ignition temperatures. The use of quartz powder in the sample to protect the boat and to assist in the release of carbon dioxide was foregone because of the high blank difficulties which seemed to result from its use.

Due to the protection afforded by the quartz sleeves which must be replaced after every 10 to 15 analyses, combustion tube mortality is low. One tube lasted for more than 150 analyses burning an average of 100 mg. of NaK each sample. Eventually, however, the fluxing action of hot alkali, and the repeated heating and cooling embrittle the quartz which then becomes porous a condition which becomes evident by observing blank values.

LITERATURE CITED

(1) Johnston, John, *Z. physik. Chem.*, **62**, 330 (1928).
(2) Jones, I. B., "Laboratory Manual of Microchemistry," Burgess Publishing Co., Minneapolis, Minn., 1941.
(3) Lebeau, M. P., *Ann. chim. et phys.*, **6**, 422 (1905).
(4) Niederl, J. B., and Niederl, Victor, "Micromethods of Quantitative Organic Analysis," 2nd ed., Wiley, New York, 1942.
(5) Phillips, J. H., "Manhattan Project Report A2912," Vol. 3, pp. 99–104, January 1946; Report A1076, Vol. 10, pp. 1–5, Feb. 16, 1946.
(6) Pregl, Fritz, "Quantitative Organic Microanalysis," 5th English ed., by Julius Grant, Blakiston, Philadelphia, 1951.
(7) Walters, S. L., and Miller, R. R., IND. ENG. CHEM., ANAL. ED., **18**, 468 (1946).

RECEIVED for review August 20, 1954. Accepted December 29, 1954. Presented before the Division of Analytical Chemistry at the 124th Meeting of the AMERICAN CHEMICAL SOCIETY, Chicago, Ill.

Ferricyanide Titration of Cobalt Using Ethylenediamine

HARVEY DIEHL and JOHN P. BUTLER[1]

Department of Chemistry, Iowa State College, Ames, Iowa

The titration of cobalt in ammoniacal solution with potassium ferricyanide has been modified by the replacement of ammonia by ethylenediamine. The cobalt(II)–ethylenediamine complex is a strong reducing agent and its potentiometric titration involves a much larger break than that with the corresponding ammonia complex. The potentiometric determination of cobalt, either directly or by back-titration, is successful if ethylenediammonium sulfate is present as well as ethylenediamine and if oxygen is rigidly excluded. Cobalt and manganese can be determined successively on the same sample except when large amounts of iron are present. The method is applicable to cobalt-bearing stainless steels and bronzes and to Stellite.

THE ferricyanide titration of cobalt was first reported by Tomicek and Freiberger (*8*) and Dickens and Maassen (*5*). In an ammoniacal solution cobalt(II) salts are oxidized quantitatively by potassium ferricyanide and the reaction can be followed potentiometrically. The break in potential at the equivalence point is about 0.2 volt. Ammonium salts have a beneficial effect on the titration, and the recommended procedure calls for addition of excess ferricyanide and back-titration with cobalt(II) nitrate. Citrate or tartrate is added to prevent the precipitation of various other metal hydroxides. The titration can then be run in the presence of iron, nickel, and copper. Manganese interferes seriously.

Several papers have dealt with this titration (*1, 4, 6, 10*). In general, it has proved far superior to the other volumetric methods for cobalt and it has been widely adopted for the determination of cobalt in ferrous and nonferrous alloys. The completeness of the reaction has been disputed (*1, 10*), and the quantitative aspects of the reaction of manganese with ferricyanide under the conditions of the titration appear unsettled. Some disagreement appears in the literature also as to the interference of chromium, either in the tri- or the sexivalent state. In any case, the potential break is not as great as might be desired and becomes even smaller with increasing amounts of cobalt (*1*).

The present paper is concerned with a modification of this titration in which ammonia is replaced by ethylenediamine. Such a titration in the presence of ethylenediamine was carried out by Bjerrum (*2*) in connection with his work on the formation constants of cobalt complexes, but no detailed study of the reaction or of applications was made.

The cobalt(II)–ethylenediamine complex is a stronger reducing agent by some 0.5 volt than the corresponding cobalt(II)–ammonia complex; the potential break at the equivalence point in the titration is thus augmented by a similar amount. The determination can be carried out as a direct titration in most instances. Using a suitable complexing agent, cobalt can be determined in the presence of manganese. Rigid precautions must be taken to avoid the untoward effects of air.

Various other amines were investigated for possible use in this work: triethylenetetramine, propylenediamine, diethylenetriamine, trimethylenediamine, and tetraethylenepentamine. The best titration was obtained with ethylenediamine.

The cobalt(II)–ethylenediamine compound is a powerful reducing agent, its standard reduction potential approaching that of the chromium(III)–chromium(II) system. Oxygen must be removed from the solution prior to the addition of ethylenediamine. This is conveniently accomplished by passing oxygen-free nitrogen through the solution to be titrated.

As in the titration using ammonia, the presence of the salt as well as the free amine is essential. If ethylenediamine alone is present, the potential drifts seriously; when both free diamine and its salt are present, the potential rapidly reaches a steady state. The ethylenediammonium salt is conveniently formed in situ by neutralizing the acid remaining after dissolution of the sample.

EXPERIMENTAL

Reagents. ETHYLENEDIAMINE. Commercial ethylenediamine was distilled over sodium hydroxide, the fraction boiling at 115–116° C. being collected.

STANDARD POTASSIUM FERRICYANIDE. Approximately 0.01*N* potassium ferricyanide was prepared by dissolving 6.6 grams of reagent potassium ferricyanide in 2 liters of water. The solution was placed in a Machlett buret and was freed of oxygen by passing nitrogen through it for 4 hours. It was then standardized against cobalt(II) sulfate.

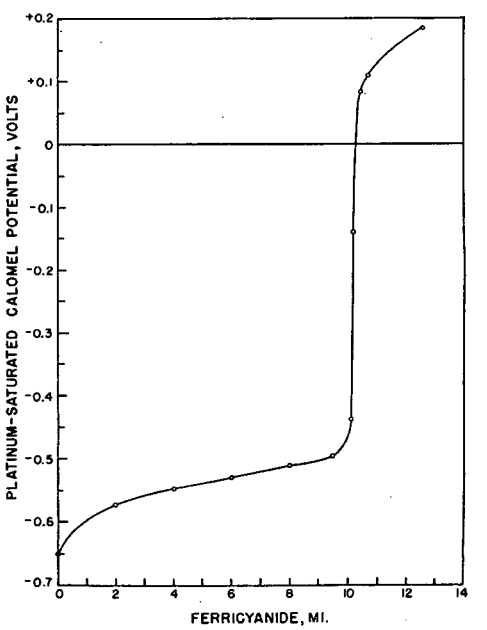


Figure 1. Titration of cobalt(II) sulfate with ferricyanide in presence of ethylenediamine and ethylenediammonium sulfate

COBALT(II) SULFATE. The reference standard, anhydrous cobalt(II) sulfate, was prepared from reagent grade cobalt(II) sulfate in the following manner. Cobalt(II) sulfate was first converted to hexamminocobaltioxalate (*3*). The luteo oxalate was then ignited at a dull red heat to cobalt(III) oxide. The oxide was dissolved in sulfuric acid and the solution filtered and concentrated. Hydrated cobalt(II) sulfate was allowed to crystallize and was then filtered off, dried, and finally heated to 555° C. (*9*). A master solution containing 15.7114 grams of this anhydrous salt per liter was prepared; aliquots were diluted as required. A solution of this salt, approximately 0.01*N* for use in back-titration procedures, was also prepared, placed in a Machlett buret, and freed of oxygen by the passage of nitrogen.

Apparatus. A Beckman Model G pH meter was used as a potentiometer. Bright platinum and saturated calomel electrodes (SCE) were used. The titrations were performed in a

[1] Present address, Institute of Paper Chemistry, Appleton, Wis.

three-necked vessel having a gas inlet tube sealed in near the bottom.

Stability of Potassium Ferricyanide Solutions. The 0.01*N* solution of potassium ferricyanide decomposed slowly on standing; a decrease in normality of 12% was observed after 46 days' storage without exposure to air; 0.1*N* solutions decomposed at a somewhat faster rate. Exclusion of light improved the stability. In any case it was found best to standardize the ferricyanide solution about the time it was used.

Standardization of Ferricyanide Solution. An aliquot of a standard solution of cobalt(II) sulfate containing 20 to 30 mg. of cobalt was pipetted into the titration cell. A volume of 2 ml. of dilute sulfuric acid (1 to 1) was added and the solution diluted to about 35 ml. Nitrogen, freed of oxygen by passage through a solution of vanadous sulfate (7), was bubbled through the titration vessel for 10 minutes. A volume of 2.0 ml. of ethylenediamine was then added and the solution titrated potentiometrically with 0.01*N* potassium ferricyanide. A representative titration curve so obtained is shown in Figure 1.

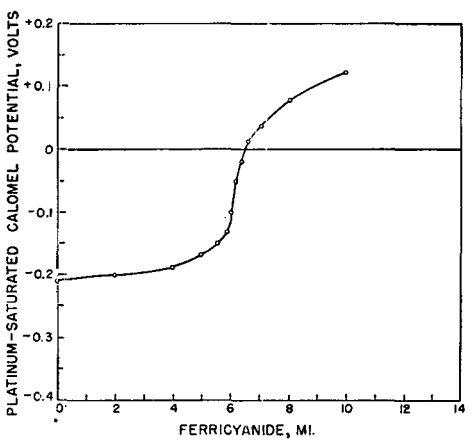


Figure 2. Titration of cobalt in presence of a large amount of iron, salicylate, and ethylenediamine

Data taken from actual determination of cobalt in stainless steel

Determination of Cobalt. Effect of Other Metals. The determination was carried out using the same procedure as for the standardization of ferricyanide. The cobalt was determined by direct titration as above or an excess of standard ferricyanide was added and the excess back-titrated with standard cobalt(II) sulfate. In either method the potential came to equilibrium quickly and the potential break was large. The direct titration was the more convenient and is recommended except for cases in which cobalt is determined in the presence of silver or a three-component mixture of chromate, vanadate, and molybdate or tungstate as noted below. The back-titration must be used in these cases.

Iron. In the presence of iron it was necessary to add a complexing agent to prevent the precipitation of iron(III) from alkaline solution. Citrate, tartrate, and sulfosalicylate were found satisfactory for this purpose and did not interfere of themselves in the cobalt determination. Pyrophosphate, fluoride, and mannitol were unsatisfactory.

The presence of iron(III) changed the potential at the start of the titration, shifting the single electrode potential of the platinum indicator electrode in the positive direction (reduction potential basis) by an amount depending on the amount of iron present. In the case of steel samples in which about 0.5 gram of iron was present, the initial potential was about −0.2 volt toward the saturated calomel electrode, as compared to −0.6 volt in the absence of iron. It was apparent that iron(III) was reduced by the cobalt(II)–ethylenediamine ion and that the subsequent titration was actually the titration of a mixture of

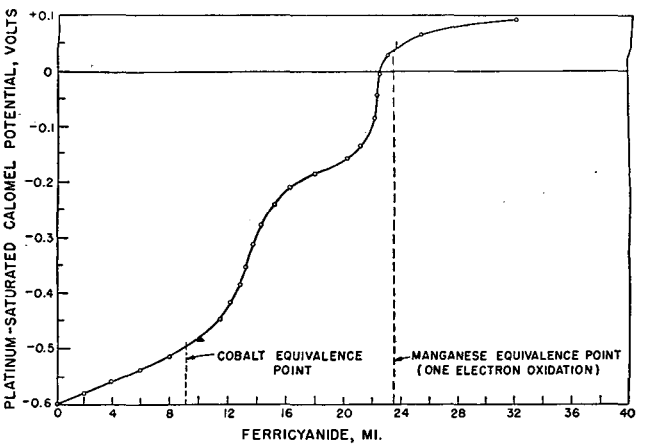


Figure 3. Titration of cobalt and manganese in presence of ethylenediamine and tartrate

iron(II) and cobalt(II) ions. This was confirmed by the titration of a weighed quantity of iron(II) ethylenediammonium sulfate under the same conditions as prevailed in the steel analysis but in the absence of cobalt. The titration was quantitative, and the potential break was approximately 0.6 volt or about the same size as that for cobalt. In the presence of ethylenediamine and citrate, tartrate, or sulfosalicylate and iron(II) compared in strength as a reducing agent to the cobalt(II)–ethylenediamine system. Accordingly, oxidation of iron(II) and cobalt(II) would be expected to occur concurrently, and such was found to be true in practice. The large, positive shift of the potential of the indicator electrode may be seen in Figure 2, which shows a typical titration curve for the determination of cobalt in stainless steel. The sulfosalicylic acid complex of iron forms at a measurable rate, and several minutes should be allowed for this reaction to occur before beginning the titration.

Nickel and Copper. In the presence of citrate or sulfosalicylate, neither nickel nor copper interfered with the titration (Table I).

Table I. Effect of Other Metal Ions on Direct Titration of Cobalt with Ferricyanide in Ethylenediamine Solution

Cobalt, Mg.		Other Metal Ion Present, Mg.	Complexing Agent
Taken	Found		
11.94	11.94	Ni, 19.05	Citrate
	11.90	Ni, 19.05	Sulfosalicylate
11.94	12.02	Cu, 20.3	Citrate
	11.98	Cu, 20.3	Sulfosalicylate
11.94	11.94	Fe(III), 10.3	Citrate
	11.87	Fe(III), 10.3	Sulfosalicylate
11.94	17.0	Mn(II), 17.7	Citrate
	11.92	Mn(II), 17.7	Sulfosalicylate
5.97	5.91	Mn(II), 208	Sulfosalicylate

Manganese. In an ethylenediamine solution, manganese(II) was oxidized by ferricyanide to manganese dioxide. When cobalt and manganese were both present in the solution, the titration proceeded in two stages; first the cobalt(II) was oxidized and then the manganese(II) salt was oxidized to the dioxide. However, the oxidation of the manganese took place slowly as the equivalence point was approached, and the exact position of the end point depended on the rate of addition of ferricyanide.

Manganese was partially oxidized to the trivalent state in an ethylenediamine solution containing citrate. The oxidation of manganese was incomplete and the potential of the manganese(III)–manganese(II) couple present was such that the

titration curve overlapped that of cobalt. A similar behavior was observed in the presence of tartrate; the titration of cobalt alone was satisfactory, but in the presence of manganese some manganese(II) tartrate was oxidized concomitantly with the cobalt and the results were not stoichiometric for either cobalt or manganese. A titration curve obtained starting with cobalt(II) and manganese(II) sulfates is shown in Figure 3.

Pyrophosphate, fluoride, and mannitol were found even poorer as complexing agents for the successive titration of cobalt and manganese. The potential drifted extensively during the titration. Neither osmium tetroxide nor potassium iodide catalyzed these reactions.

The back-titration procedure did not work in the presence of manganese. The potential drifted seriously and reduction of the excess ferricyanide and of the manganese-citrate or manganese-tartrate complex took place concurrently.

The interference of manganese in the cobalt titration was finally obviated by use of sulfosalicylic acid. Both cobalt(II) and manganese(II) were oxidized, the quantitative oxidation of cobalt taking place first. The manganese was subsequently oxidized quantitatively to the trivalent state. The cobalt end point was excellent; that for manganese was less sharp (Figure 4). The cobalt titration was satisfactory for ratios of manganese to cobalt of up to 40 to 1. The successive titration of cobalt and manganese failed, however, in the presence of large amounts of iron. A large amount of iron(III) shifted the initial potential in the positive direction to such an extent that the break at the manganese end point disappeared (Figure 5). Amounts of iron up to four or five times that of the manganese could be tolerated.

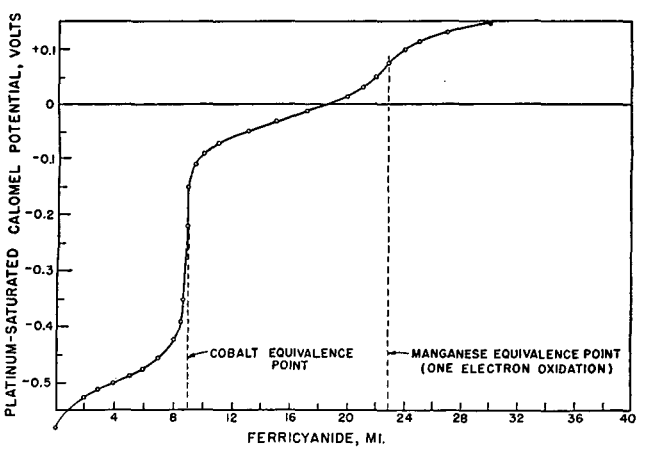


Figure 4. Titration of cobalt and manganese with ferricyanide in presence of ethylenediamine and sulfosalicylate

CHROMIUM, MOLYBDENUM, VANADIUM, AND TUNGSTEN. Direct Titration. Chromium(VI), molybdenum(VI), and tungsten(VI), and vanadium(V) interfered in the direct titration of cobalt with ferricyanide in an ethylenediamine-citrate solution. The cobalt(II)–ethylenediamine system initially present reduced the higher valent forms of these elements to lower valent forms; reoxidation by the ferricyanide proceeded slowly and was not complete.

The interference of chromate in the direct titration of cobalt was obviated by reducing the chromate to chromium(III) ion which did not interfere. This reduction was conveniently effected by adding hydrogen peroxide to an acid solution of the chromate and then boiling to destroy the excess peroxide. The peroxide reduction could not be used if tungsten or molybdenum were present, owing to the formation of stable peroxyacids of these elements which interfered with the subsequent titration. When these elements were present it was necessary to use the back-titration method.

Back-Titration. In the back-titration procedure, chromate, tungstate, molybdate, and vanadate did not interfere in the determination of cobalt either when present singly or when present in pairs. However, when a three-component mixture, chromate-molybdate-vanadate, chromate-tungstate-vanadate, or chromate-molybdate-tungstate, was present (as in National Bureau of Standards 153 steel), the back-titration scheme also failed unless the acidity of the solution was adjusted prior to the addition of

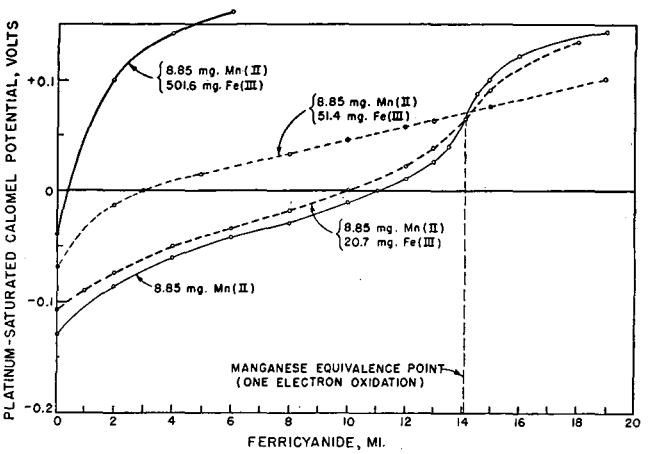


Figure 5. Effect of iron(III) on titration of manganese with ferricyanide in ethylenediamine-sulfosalicylate solution

Table II. Effect of Other Metal Ions on Back-Titration of Ferricyanide with Cobalt(II) Sulfate in Ethylenediamine Solution

Cobalt Equivalent of Ferricyanide Taken, Mg.	Cobalt Required for Back-Titration, Mg.	Other Metal Ion Present, Mg.	Complexing Agent
13.25	13.30	Cr(VI), 2.84	Citrate
	13.42	Cr(VI), 2.84	Sulfosalicylate
13.25	13.13	V(V), 19.7	Citrate
	13.42	V(V), 19.7	Sulfosalicylate
13.25	13.25	Mo(VI), 32.9	Citrate
	13.25	Mo(VI), 32.9	Sulfosalicylate
13.25	12.42	Ag, 22.3	Citrate
	12.84	Ag, 22.3	Sulfosalicylate
13.25	13.40	W(VI), 85.8	Citrate
	13.25	W(VI), 85.8	Sulfosalicylate
13.30	13.24	V(V), 19.7 Cr(VI), 2.84	Citrate
13.30	13.24	V(V), 19.7 W(VI), 85.8	Citrate
13.30	13.20	V(V), 19.7 Mo(VI), 65.8	Citrate
13.30	13.37	W(VI), 85.8 Mo(VI), 65.8	Citrate
13.30	13.24	Cr(VI), 2.84 W(VI), 85.8	Citrate
13.30	13.34	Cr(VI), 2.84 Mo(VI), 65.8	Citrate
13.30	13.20	Cr(VI), 2.84 Mo(VI), 65.8 V(V), 19.7	Citrate
13.30	13.09	Cr(VI), 2.84 W(VI), 85.8 V(V), 19.7	Citrate
13.30	13.27	Cr(VI), 2.84 W(VI), 85.8 Mo(VI), 65.8	Citrate
13.30	13.20	V(V), 19.7 W(VI), 85.8 Mo(VI), 65.8	Citrate
13.30	13.27	V(V), 19.7 Cr(VI), 2.84 Mo(VI), 65.8 W(VI), 85.8	Citrate

citrate. The three-component system tungstate-molybdate-vanadate did not interfere in the back-titration method.

The reduction potentials of chromate, molybdate, tungstate, and vanadate were greatly reduced on decreasing the acidity of the solution; the effect was so great that in alkaline solution ferricyanide was the stronger oxidizing agent. That only the three-component systems mentioned caused trouble in the determination of cobalt by the back-titration method implied that a heteropoly acid was present which is a fairly powerful oxidizing agent, sufficient to oxidize citric acid at low pH (Table II).

A number of attempts were made to apply the back-titration procedure to the determination of cobalt in NBS 153 steel. This steel contains 0.219% manganese in addition to macro amounts of cobalt, tungsten, vanadium, chromium, and molybdenum and is as complex as any likely to be encountered in practice.

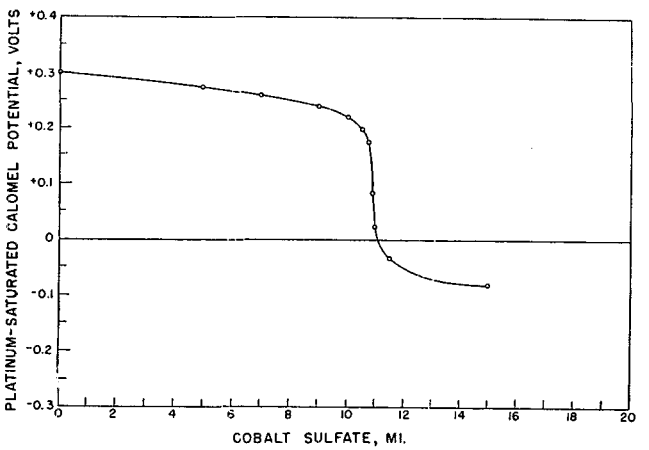


Figure 6. Back-titration of excess ferricyanide with 0.01*N* cobalt(II) sulfate

Silver present. Data taken from analysis of copper-beryllium-silver alloy

In the course of analysis of NBS 153, the following effects were investigated: method of dissolving the sample, use of peroxide to reduce chromium(VI), preliminary separations, choice of compexing agent for iron(III), control of pH at the start of the titration, effect of oxygen diffusion into the solution during the titration, and possible inhomogeneity of the sample.

The best acid mixture for dissolving the sample consisted of equal volumes of nitric and hydrochloric acids. The solutions were fumed with perchloric acid to remove nitric acid and to oxidize all the constituents of the steel to their higher-valent forms. Use of peroxide to reduce chromium(VI) was ruled out by the presence of tungsten and molybdenum. Other reducing agents such as sulfur dioxide, formaldehyde, formic acid, oxalic acid, bisulfite, and sodium azide were found effective, but other constituents of the sample were reduced at the same time. In any case, direct titration of the cobalt(II) ethylenediamine system with ferricyanide was ineffective following reduction of chromium.

Direct titration of cobalt(II) with ferricyanide following volatilization of chromium as chromyl chloride gave erratic results. Removal of tungsten and molybdenum by precipitation with α-benzoin oxime failed to improve the reproducibility of the cobalt determination. As the procedure of Dickens and Maassen is applicable to NBS 153 steel without preliminary separations, further separation studies were deemed inadvisable.

Choice of complexing agent for iron(III) lay between citrate and sulfosalicylate. The latter could not be used in either the direct or back-titration procedures; apparently sulfosalicylic acid is oxidized by the chromate-molybdate-tungstate system and analysis for cobalt(II) in the resultant solution was ineffectual. Use of citrate was thus necessary in spite of the suspected interference of manganese.

Citric acid was oxidized by the chromate-tungstate-molybdate system at low pH. When the pH of the solution was adjusted to 2.0 to 2.5 prior to the addition of sodium citrate, oxidation of citrate was minimized.

Oxygen was rigorously excluded from the solution at all times. In addition to sweeping nitrogen through the solution to be titrated, the solutions were freed of dissolved oxygen prior to their use in the course of the titration.

Inhomogeneity of the sample was suspected when concordant results were not obtained. A master solution of NBS 153 steel was made up, and aliquots were withdrawn for titration. Concordant results could not be obtained using aliquots, although the back-titration with ferricyanide gave results ranging from 8.1 to 8.9% cobalt. (The NBS value was 8.45%.)

The failure of the present method in the case of NBS 153 steel may be due to the presence of manganese, but one of the other constituents or a combination of several may actually be the culprit. The procedure that was finally worked out gives an approximation of the cobalt content of NBS 153 steel.

SILVER. It was not possible to determine cobalt by direct titration with ferricyanide in the presence of silver. The cobalt(II)–ethylenediamine complex reduced the silver to the metal and the metallic silver redissolved slowly during the titration with ferricyanide, leading to spurious results for cobalt.

The back-titration of excess ferricyanide with cobalt(II) sulfate was successful in the presence of silver (Figure 6). It was necessary to add the ferricyanide just before adding the ethylenediamine; oxidizing conditions thus prevailed at the start of the titration. The reduction of the excess ferricyanide took place before reduction of the silver occurred, the titration curve showing two breaks. However, the reduction of silver was sluggish, and the second part of the titration was of little use as an analytical method.

APPLICATION TO METALLURGICAL MATERIALS

The proposed method was applied to a number of metallurgical products: beryllium-cobalt bronze, silver absent (direct titration, Table III), beryllium-cobalt bronze containing silver (back-titration, Table IV), stainless steel (direct titration, Table V), chrome-vanadium-molybdenum steel, NBS 153 (back-titration, Table VI), and Haynes Stellite (Table VII).

In general, the direct titration is applicable, the exceptions

Table III. Determination of Cobalt in Bronze (Silver Absent)[a]

Alloy and Reported Analysis	Weight of Sample, Grams	Standard $K_3Fe(CN)_6$ Used, Ml.	Cobalt Found, %
Be-Cu-Co alloy A (cobalt 0.53% as determined spectrographically)[b]	1.0847	6.45	0.43
	1.1124	6.50	0.43
	1.0449	6.30	0.44
	1.0868	6.51	0.44
	1.1985	7.48	0.45
Be-Cu-Co alloy B (cobalt 0.27% as determined spectrographically)[b]	1.2409	4.6	0.27
	1.1378	4.0	0.26
	1.0667	4.2	0.29
	0.9667	3.6	0.27
	1.2041	4.7	0.29
Be-Cu-Co alloy C (cobalt 0.33% as determined spectrographically)[b]	0.9761	4.8	0.36
	1.2502	6.3	0.37
	0.9702	4.6	0.35
	1.1519	5.7	0.37
	1.0803	5.3	0.36
Ampco No. 91 bronze (cobalt 2.897%)	0.3409	14.35	2.92
	0.3868	16.5	2.97
	0.3361	13.7	2.83
	0.1992	8.35	2.91
	0.4180	17.62	2.93

[a] Results given for consecutive analyses on each sample.
[b] Spectrographic analysis of beryllium-copper-cobalt alloys showed presence of beryllium, 1.0 to 2.25%; tin, 0.05 to 0.40%; and small amounts of iron, silicon, aluminum, lead, zinc, and nickel.

Table IV. Determination of Cobalt in Silver-Bearing Bronze

[Back-titration of excess ferricyanide with cobalt(II) sulfate[a]]

Weight of Sample, Gram	Standard $K_3Fe(CN)_6$ Added, Ml.	Standard $CoSO_4$ Used, Ml.	Cobalt Found, %
0.4978	25	13.6	1.73
0.5941	25	10.4	1.77
0.7020	25	7.80	1.73
0.4568	25	14.2	1.79
0.6228	25	9.90	1.75
		Average	1.75

[a] Analysis of beryllium-copper-cobalt alloy D. Cobalt, 1.71%; beryllium, 0.25 to 0.50%; silver, 0.90 to 1.10%. Also small amounts of iron, silicon, aluminum, tin, lead, zinc, and nickel.

Table V. Determination of Cobalt in Armco Stainless Steel No. 29 by Direct Titration[a]

Weight of Sample, Gram	Standard $K_3Fe(CN)_6$ Used, Ml.	Cobalt Found, %
0.6603	6.5	0.64
0.8017	7.3	0.60
0.7273	6.6	0.59
0.7429	7.1	0.63
0.6653	6.4	0.63
	Average	0.62

[a] Reported analysis. Chromium, 18%; nickel, 10%; manganese, 1.20 to 1.35%; cobalt, 0.60 to 0.65%. Some niobium also present.

being ferrous alloys containing chromium, vanadium, and either tungsten or molybdenum and silver-bearing cobalt bronze. In these cases the back-titration procedure must be used.

RECOMMENDED PROCEDURES

Cobalt in Bronze (Silver Absent). Weigh accurately a sample of no more than 1 gram and of such size as to contain 5 to 30 mg. of cobalt. Dissolve the sample in the minimum amount of nitric acid; 4 ml. are adequate for a 0.3-gram sample. Add 5 ml. of perchloric acid and evaporate the solution to strong fumes over a burner. Cool the mixture, dilute to 30 ml., and boil for 5 minutes. Cool again and transfer to the titration vessel. Bubble oxygen-free nitrogen through the solution for 10 minutes. Add ethylenediamine, 2.5 ml. for a 0.3-gram sample, 5.0 ml. for a 1-gram sample. Titrate the sample with 0.01*N* ferricyanide and follow the titration potentiometrically using a platinum–saturated calomel electrode system.

Cobalt in Bronze (Silver Present). Weigh accurately a sample of 0.5 gram or of such size as to contain 5 to 30 mg. of cobalt. Dissolve the sample in a mixture of 5 ml. of nitric acid and 5 ml. of perchloric acid, and evaporate the solution to strong fumes of perchloric acid. Cool the mixture and dilute to 30 ml. Boil the clear solution for 10 minutes; if a precipitate of silver chloride appears at this stage, the sample must be discarded. After cooling, transfer the solution to the titration vessel and bubble oxygen-free nitrogen through the solution for 10 minutes. Add concentrated sodium hydroxide solution dropwise to the point of incipient precipitation, and then add 5 grams of sodium citrate. In rapid succession add an excess of standard ferricyanide and 2 ml. of ethylenediamine. Titrate the solution with a standard cobalt(II) sulfate solution, following the titration potentiometrically. The ferricyanide should be standardized against cobalt(II) sulfate under the same conditions.

Stainless Steel. Weigh accurately a sample of less than 1 gram and of such size as to contain 5 to 30 mg. of cobalt. Dissolve the sample in a mixture of 5 ml. of nitric acid and 10 ml. of hydrochloric acid, using gentle heat as necessary. Add 6 ml. of perchloric acid and evaporate the solution to strong fumes of perchloric acid. Cool the mixture and dilute to 30 ml. with water. Add 4 ml. of 15% hydrogen peroxide and boil the solution for 10 minutes to reduce the volume and destroy the excess peroxide. Transfer the resulting blue solution to the titration vessel and add 3 grams of sulfosalicylic acid. Bubble nitrogen through the solution for 15 minutes to ensure the removal of oxygen. Add 5 ml. of ethylenediamine and titrate the sample with 0.01*N* ferricyanide, determining the end point potentiometrically.

Chrome-Vanadium-Molybdenum Steel (such as National Bureau of Standards 153). Weigh accurately a sample of 0.1 to 0.2 gram and dissolve in a mixture of 5 ml. of nitric acid and 5 ml. of hydrochloric acid. Add 5 ml. of perchloric acid and evaporate to strong fumes over a burner. Cool the mixture, dilute to 30 ml., and boil for 10 minutes. Cool again and transfer the solution to the titration vessel. Add concentrated sodium hydroxide dropwise to the solution until a pH of 2.0 to 2.5 is reached. Bubble nitrogen through the solution for 10 minutes. Then add in rapid succession 5 ml. of a saturated sodium citrate solution, an excess of ferricyanide (25 ml. in the case of NBS 153 steel samples), and 2.5 ml. of ethylenediamine. Titrate the solution with standard cobalt(II) sulfate, following the titration potentiometrically.

Table VI. Determination of Cobalt in National Bureau of Standards Cobalt-Molybdenum-Chromium Steel, Standard Sample 153[a]

Weight of Sample, Gram	Standard $K_3Fe(CN)_6$ Added, Ml.	Standard $CoSO_4$ Used, Ml.	Cobalt Found, %[b]
0.1484	20	6.90	8.42
0.1484	20	6.90	8.42
0.1484	20	6.70	8.51
0.1484	20	6.60	8.57
0.1484	20	4.70	8.83

[a] Consecutive analyses on aliquot samples. Back-titration of excess ferricyanide with cobalt(II) sulfate.
[b] Reported by NBS. 8.45% cobalt.

Table VII. Determination of Cobalt in Haynes Stellite[a]

Wt. Sample (Aliquot), Gram	Ferricyanide, Ml.	Cobalt, %
0.0419	26.25	58.3
0.0419	29.3	58.4
0.0419	29.4	58.5
0.0419	29.35	58.4
0.0421	29.4	58.3
0.0421	29.5	58.5
0.0421	29.7	58.7
0.0421	29.7	58.7
0.0421	29.4	58.3
	Average	58.5

[a] Reported. 58.73% cobalt.

Stellite. Weigh accurately a sample of about 2 grams and dissolve in 60 ml. of hydrochloric acid over low heat. Add 50 ml. of perchloric acid and heat to strong fumes of perchloric acid over a burner. Cool the mixture, dilute to 150 ml., and add 10 ml. of 15% hydrogen peroxide. Boil the solution to destroy excess peroxide, cool, and dilute to 1 liter in a volumetric flask. Withdraw an aliquot containing 5 to 30 mg. of cobalt and transfer to the titration vessel. Bubble nitrogen through the solution for 15 minutes, and then add 2 grams of sulfosalicylic acid and 2.5 ml. of ethylenediamine, in that order. Titrate the solution with ferricyanide, determining the end point potentiometrically.

ACKNOWLEDGMENT

The authors wish to thank Arba Thomas, Armco Steel Corp., R. H. Van Tyne, Firth Sterling Steel Co., G. D. Kriebel, Beryllium Co., and Albert M. Reith, Haynes Stellite Co., for generously providing the samples used in this study. J. P. Butler is indebted to the Shell Oil Co. for financial aid in the form of a fellowship which enabled the completion of this work.

LITERATURE CITED

(1) Bagshawe, B., and Hobson, J. D., *Analyst*, **73**, 152 (1948).
(2) Bjerrum, J., "Metal Ammine Formation in Aqueous Solution," p. 269, P. Haase and Son, Copenhagen, 1941.
(3) Bjerrum, J., and McReynolds, J. P., "Inorganic Syntheses," Vol. II, p. 216, McGraw-Hill Book Co., New York, 1946.
(4) Chirnside, R. C., Cluley, H. J., and Proffitt, P. M. C., *Analyst*, **72**, 351 (1947).
(5) Dickens, P., and Maassen, G., *Arch. Eisenhüttenw.*, **9**, 487 (1935).
(6) Hall, A. J., and Young, R. S., *Chemistry & Industry*, **24**, 394 (1946).
(7) Meites, L., and Meites, T., ANAL. CHEM., **20**, 984 (1948).
(8) Tomicek, O., and Freiberger, F., *J. Am. Chem. Soc.*, **57**, 801 (1935).
(9) Willard, H. H., and Hall, D., *Ibid.*, **44**, 2219 (1922).
(10) Yardley, J. T., *Analyst*, **75**, 156 (1950).

RECEIVED for review October 11, 1954. Accepted December 30, 1954.

1-(2-Pyridylazo)-2-naphthol as a Possible Analytical Reagent

KUANG LU CHENG[1] and ROGER H. BRAY

University of Illinois, Urbana, Ill.

The dye, 1-(2-pyridylazo)-2-naphthol, forms various colored metal chelates. Their stability is greatly affected by the acidity. The alkali and alkaline earth metals do not form colored chelates with the dye. The dye may be used as an indicator in the complexometric titration of zinc, copper, cadmium, etc., with ethylenediaminetetraacetic acid. Among the metals reacting with the dye, most form reddish colored chelates; cobalt and palladium form greenish colored chelates. The effect of masking agents on the formation of colored chelates has been investigated. Among the metals tested, ethylenediaminetetraacetic acid prevents all metals from reacting with the dye. Fluoride and citrate prevent iron, bismuth, lead, and thorium from reacting. In the presence of cyanide only bismuth and lead react with the dye.

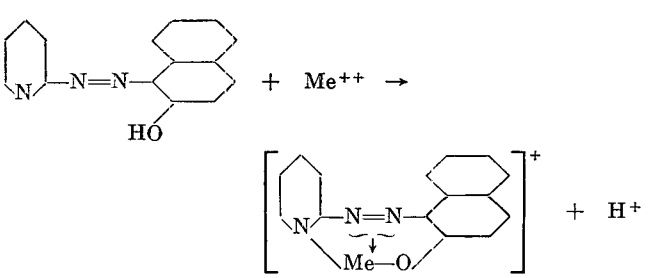
IN 1915 and 1918 Tschitschibabin (*9, 10*) reported the synthesis of some *o*-hydroxyl compounds. Liu (*4*) found that one of these, 1-(2-pyridylazo)-2-naphthol (a dye), formed chelates with many heavy metals. The possibility of using this dye for analytical purposes was investigated. Further studies are needed.

The dye is an orange-red amorphous substance, nearly insoluble in water but soluble in a variety of organic solvents to which it imparts a yellow color.

It was prepared (*4*) by coupling 2-naphthol with pyridyl diazotate in absolute alcohol with slow passing of carbon dioxide. The diazotate was prepared by adding a solution of sodium ethylate to a mixture of 1-aminopyridine and butyl nitrite under reflux.

CHELATE FORMATION

The chelate compounds are easily prepared by adding a few drops of a solution of the dye in methanol to solutions of heavy metals. The reaction may be written as follows:



The results of the experiments indicated that the pink or red colored chelate compounds were formed regardless of the nature of the acid radical, but the pH of the solution was of great importance in their reaction. The solutions containing heavy metals showed a red color under slightly acid, neutral, or alkaline conditions.

The following ions failed to give a detectable precipitate or coloration: aluminum, calcium, magnesium, strontium, barium, antimony, chromium(VI), potassium, sodium, lithium, titanium, zirconium, mercury(I), germanium, tellurium, ruthenium, rhodium, iridium, cesium, beryllium, osmium, arsenate, arsenite, nitrite, nitrate, chloride, bromide, iodide, fluoride, sulfate, sulfite, perchlorate, phosphate, tungstate, molybdate, bromate, borate, acetate, citrate, tartrate, oxalate, cyanide, thiosulfate, cyanate, thiocyanate, and malonate.

The ions that gave a color or precipitate with the dye are listed in Table I. The tests were made by adding 1 drop of 0.1% dye solution in methanol to 2 ml. of 0.1 mg. of metal per ml. of the solution to be tested. The test tube was shaken and a reddish color formed immediately. When allowed to settle for a while, a precipitate appeared. This precipitate could be extracted by shaking it with about 3 ml. of amyl alcohol or carbon tetrachloride.

All the ions listed in Table I formed red chelates with the dye, except cobalt and palladium(II), which formed a green precipitate when allowed to stand.

Most of the colored chelates were soluble in amyl alcohol; the chelates of nickel, cobalt, cadmium, and zinc were soluble in both amyl alcohol and carbon tetrachloride.

APPLICATIONS

The dye may be applied in complexometric titrations as an indicator. Because it forms colored chelates with some metals, it may also be used as an organic reagent for colorimetric determinations or spot tests.

As an Indicator in Complexometric Titration. TITRATION OF ZINC. The solution containing from 5 to 25 mg. of zinc, previously adjusted to pH 5 to 7 with sodium acetate, was titrated

Table I. Color Reaction of Metallic Ions with 1-(2-Pyridylazo)-2-naphthol

Ion	Color of Water	Color of Amyl Alcohol	Color of CCl_4	Limit of Sensitivity, γ	Remarks
H_2O	Yellow	Yellow	Yellow		
Bi(III)	Pink	Yellow	Pink	4	Partly soluble in CCl_4; pink color disappeared when amyl alcohol was added.
Cd(II)	Red	Red	Red	0.5	Yellow in amyl alcohol, but turned red when NaOAc was added.
Cu(II)	Deep red	Red	Yellow	0.2	Slightly soluble in CCl_4 at high concentration
Pd	Green	Green	Green	4	
Pt	Red	Red			
Sn(II)	Pink	Yellow	Yellow	24	Pink color disappeared when CCl_4 or amyl alcohol was added.
UO_2(II)	Red	Red	Pink (slightly)	2	
Hg(II)	Red	Yellow	Yellow	0.4	Pink color in H_2O layer disappeared when amyl alcohol was added.
Th(II)	Orange-red	Yellow	Yellow	46	Same as Hg
Co(II)	Brownish red	Green	Green	0.4	
Pb(II)	Red	Red	Yellow	2	
Fe(II)	Red				
Fe(III)	Dark red	Red	Yellow	1	
Ni(II)	Red	Red	Red	0.4	
Zn(II)	Bright pink	Red	Red	0.2	
La(III)	Red	Red	Red	0.5	
Ce(IV)	Pink	Red			
In(II)	Red	Red		0.4	
Sc(III)	Red	Red		0.5	
Eu(II)	Red	Red		0.2	

[1] Present address, Department of Chemistry, University of Connecticut, Storrs, Conn.

with 0.1*M* disodium ethylenediaminetetraacetic acid (EDTA) using 3 to 4 drops of 0.1% dye solution in methanol as indicator. The end point was from pink to yellow.

TITRATION OF COPPER. To the nearly neutral solution containing from 5 to 25 mg. of copper were added 1 ml. of glacial acetic acid and 3 to 4 drops of 0.1% dye in methanol. The solution was then titrated with 0.01*M* ethylenediaminetetraacetic acid solution. The end point was from red to yellowish green. A violet color or blue color appeared before the end point was reached if more than a few milligrams of copper were present.

TITRATION OF CADMIUM. The titration was made in a manner similar to that described for zinc. The pH of the solution was adjusted to above 6.0.

When the dye was used as an indicator in the complexometric titration of the metals listed in Table I, the end points were sharp. The titration was subject to interference because both the dye and ethylenediaminetetraacetic acid are not specific reagents for any metal under the titration conditions. However, since this titration technique is extremely simple and very accurate, it would be useful in analysis. The solution to be titrated should be adjusted to the proper pH (see Table II) and the ethylenediaminetetraacetic acid should be standardized by a standard metal solution. The effect of pH on the color or stability of the chelates varied with different metals. The zinc chelate was found to be very sensitive to acidity. The results obtained in the determination of zinc, copper, and cadmium are shown in Table III. Other metals which form colored compound with the dye may also be titrated with ethylenediaminetetraacetic acid.

Table II. Color Stability of Metal 1-(2-Pyridylazo)-2-naphtholates and pH

Metal	Color Stability: Maximum at pH below	Color Stability: Maximum at pH above
Zn(II)	3.5	5.0
Cu(II)	0.5	1.5
Ni(II)	2.0	2.5
Cd(II)	5.0	6.5
Fe(II)	0.5	1.0
Fe(III)	1.8	2.5
Pb(II)	4.5	7.5
Th(II)	3.7	7.0
Tl(I)	10.5	11.0
Ag(I)	5.0	7.0
Hb(II)	3.0	4.5
UO_2(II)	3.5	7.0
Co(II)	3.5	5.0
Co(III)	0.1	1.0
Bi(III)	1.3	1.8

Table III. Complexometric Titration of Metals Using 1-(2-Pyridylazo)-2-naphthol as an Indicator

Metal	Titration pH	Amount Taken, Mg.	Amount Found, Mg.
Zinc	5.0	1.00	1.02
			1.04
		2.00	2.02
			1.98
		5.00	5.00
			5.02
			4.97
Copper	2.5	1.00	1.00
			1.02
		2.00	2.00
			2.00
		5.00	5.00
			5.01
Cadmium	6.0	1.00	1.03
			0.97
		2.00	2.03
			2.00
		5.00	5.02
			4.98

When the solution containing zinc was adjusted to pH 3.5, the zinc-dye chelate was not formed, but at that pH the copper-dye chelate was very stable. An attempt was made to titrate copper in the presence of zinc with ethylenediaminetetraacetic acid using the dye as an indicator. Because the stability constant of copper–ethylenediaminetetraacetic acid (log K = 18.3) is higher than that of zinc–ethylenediaminetetraacetic acid (log K = 16.1) (*5*), such a titration could be successful. But when a mixture of copper and zinc was adjusted to pH 2.0, the titration result gave the total amount of copper and zinc, instead of copper only.

As Color-Forming Agent. The highly colored chelate or lake formed with many heavy metals under various conditions was useful for the detection and determination of certain metals. These colored chelates are soluble in either isoamyl alcohol or carbon tetrachloride. Colorimetric comparison may be made by extraction with an organic solvent, or directly in an aqueous solution by adding gum arabic to prevent turbidity. The absorption spectra of the metal chelates are shown in Figure 1. The calibration curves were prepared as follows:

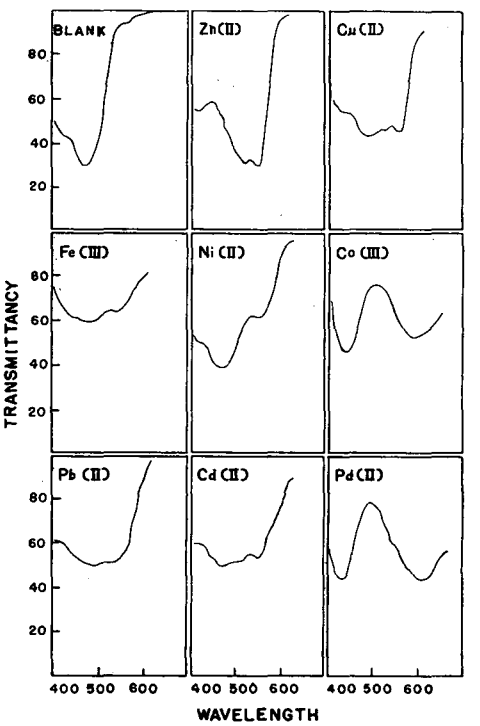


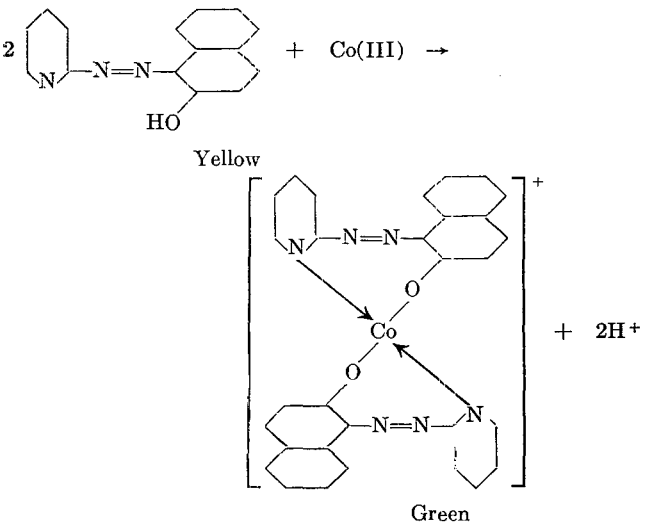
Figure 1. Absorption spectra of metal-dye chelates

ZINC, COPPER, AND NICKEL. Suitable aliquot portions of the metal solution (from 0 to 10 γ per 10 ml.) were taken into 60-ml. separatory funnels and to each were added acetic acid or sodium acetate for adjusting to the proper pH and exactly 10 ml. of 0.001% dye solution in isoamyl alcohol. Then the solutions were vigorously shaken for 3 minutes or longer. After separation and centrifugation, the isoamyl alcohol solutions were measured at 555, 560, and 560 mμ, for zinc, copper, and nickel, respectively. Beer's law was followed.

COBALT. Solutions containing 0, 2, 4, 6, 8, and 10 γ of cobalt, respectively, were pipetted into 60-ml. separatory funnels. The solution was adjusted to a pH of approximately 5. To each separatory funnel, 0.2 ml. of 0.1% dye solution in methanol, 1 drop of saturated potassium periodate solution, and then exactly 10 ml. of isoamyl alcohol were added. The funnels were vigorously shaken for more than 5 minutes. After separation and centrifugation, the isoamyl alcohol solution was measured at 640 mμ. A typical calibration curve is shown in Figure 2.

Alternatively, the measurement of cobalt was accomplished in aqueous solution as follows: To about 10 ml. of the slightly acid solution containing less than 30 γ of cobalt in a 25-ml. volumetric flask, 1 ml. of 1% gum arabic solution, and then 2 ml. of 0.1% dye solution in methanol were added. The solution was mixed and diluted to about 20 ml. with water. After approximately 5 minutes, 1 ml. of 1 to 1 hydrochloric acid was added to the solution and then it was made to volume with water. The absorbance was measured within 30 minutes at 640 mμ. The calibration curve is also shown in Figure 2. The ratio of the dye to cobalt for the green complex formation was measured by letting various amounts of the dye react with a known amount of cobalt. The results, obtained from the minimum amount of the dye required for the maximum absorbance of known amount of cobalt, showed

that 2 molecules of the dye reacted with 1 atom of cobalt. The reaction of the formation of the green cobalt chelate is probably as follows:



In the presence of not more than traces of palladium, this dye may be specific for cobalt when the red color is changed to green after the solution is acidified to pH below 1. The red cobalt(II)–dye chelate was very stable in pure methanol or ethyl alcohol; however, when a small amount of water or acid was added the red color changed to green rapidly, probably because of the rapid oxidation of cobalt(II) in the acid medium in the presence of water. The reaction *failed when the cobalt(II) solution was* acidified by concentrated hydrochloric acid before the dye was added.

An attempt was made to use the masking agents such as ethylenediaminetetraacetic acid, cyanide, fluoride, and citrate in order to detect one metal in the presence of others without separation. The experiments were conducted as follows:

One drop of 1000 p.p.m. of neutral or slightly acid metal solution was placed on the porcelain dish. One drop of the masking agent (5% aqueous solution) was added. Then 1 drop of 0.1% dye solution in methanol was added and the mixture was stirred with a small glass rod. The results are shown in Table IV.

Table IV. Effect of Masking Agents on Color Formation of Metal 1-(2-Pyridylazo)-2-naphtholates

Metal	EDTA	Sodium Cyanide	Sodium Fluoride	Sodium Citrate
H_2O	Y	O	Y	Y
Fe(III)	Y	Y-O	Y	Y
Co(III)	Y	O	G	G
Zn(II)	Y	O	P	P
Cu(II)	Y	O	W-R	V
Ni(II)	Y	O	PP	R
Bi(III)	Y	R-B	Y[a]	Y
Th(II)	Y	O	Y[a]	Y
Pb(II)	Y	W-R	Y[a]	Y
Cd(II)	Y	O	V	R
Hg(II)	Y	O	Y	V
Ag(I)	Y	O	B-R	B
Pt	Y	O	Y	Y
Pd	Y	O	G	Y
In(II)	Y	R		
Sc(III)	Y	R		
Ce(IV)	Y	R		

[a] Color turned to pink after addition of more sodium acetate. Y, yellow; O, orange; P, pink; R, red; B, brown; G, green; V, violet; R-B, reddish brown; PP, purple; B-R, brownish red; Y-O, yellowish orange; W-R, wine red.

Table V. Determination of Zinc, Copper, and Cadmium by Complexometric Titration in Presence of Foreign Metals

[1-(2-Pyridylazo)-2-naphthol as indicator]

Foreign Metal Present	Mg.	Zinc, Mg. Taken	Zinc, Mg. Found	Copper, Mg. Taken	Copper, Mg. Found	Cadmium, Mg. Taken	Cadmium, Mg. Found
Ca	10	5.00	5.00	5.00	5.00	5.00	5.00
	100	5.00	5.03	5.00	5.00	5.00	5.04
Mg	10	5.00	5.00	5.00	5.00	5.00	5.03
	100	5.00	5.01	5.00	5.01	5.00	5.01
Fe(III)[a]	10	5.00	5.03	5.00	5.00	5.00	5.04
	100	5.00	5.06	5.00	4.98	5.00	4.97

[a] One gram of sodium fluoride added before adding the dye indicator.

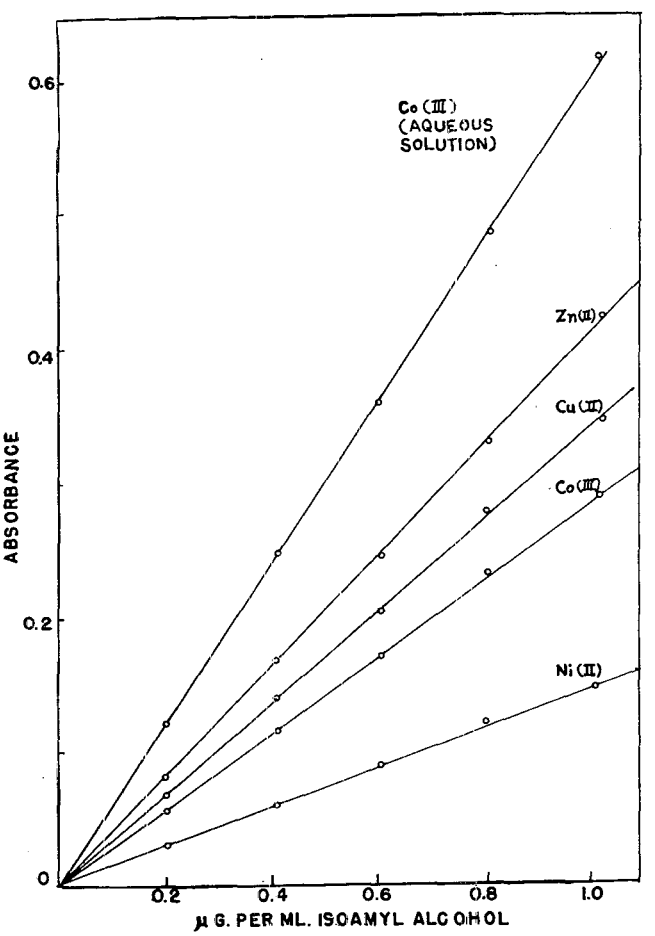


Figure 2. Calibration curves

Ethylenediaminetetraacetic acid prevented all metals tested from reacting with the dye. Cyanide prevented the metals other than bismuth and lead from reacting with the dye. Fluoride prevented iron(III), bismuth, lead, thorium, mercury(II), and platinum from reacting with the dye. Citrate had a masking effect similar to fluoride except for mercury(II). The dye formed a bright red lake with thorium in alkaline medium. The thorium-dye chelate formation was inhibited by adding sodium acetate.

DISCUSSION

Ethylenediaminetetraacetic acid has been widely used in the titration of metals. Eriochrome black T, murexide, salicylic acid, Tiron, etc., have been used in the complexometric titrations (*2, 3, 6–8*). The results obtained indicate that the dye may be used as a metal indicator in the complexometric titration of zinc, copper, cadmium, thorium, and other metals. When Eriochrome black T or murexide is used as an indicator for complexometric titration of copper, zinc, or cadmium as suggested by Biedermann and Schwarzenbach (*1*), alkaline earth metals interfere. If the dye is used as an indicator in the complexometric titration of copper, or zinc, in a slightly acid medium, the alkaline earth metals do not interfere (see Table V).

The interference by other metals has not been fully investi-

gated. In certain cases, if no interference is expected or when the interfering metals are masked by proper masking agents, the dye may prove to be useful and selective. It has been applied to the complexometric titration of zinc in Bacitracin. The tests based on the formation of green cobalt(III)-dye chelate and palladium(II)-chelate are not interfered with by other common metals.

LITERATURE CITED

(1) Biedermann, W., and Schwarzenbach, G., *Chimia*, **2**, 56–8 (1948).
(2) Cheng, K. L., Bray, R. H., and Kurtz, Touby, ANAL. CHEM., **25**, 347–8 (1953).
(3) Cheng, K. L., Kurtz, Touby, and Bray, R. H., *Ibid.*, **24**, 1640–1 (1952).
(4) Liu, J. Ch.-I., Ph.D. thesis, University of Illinois, 1951.
(5) Martell, A. E., and Calvin, M., "Chemistry of the Metal Chelate Compounds," Prentice-Hall, New York, 1952.
(6) Schwarzenbach, G., and Biedermann, W., *Helv. Chim. Acta*, **31**, 678–87 (1948).
(7) Schwarzenbach, G., and Gysling, H., *Ibid.*, **32**, 1314–25 (1949).
(8) Schwarzenbach, G., and Willi, A., *Ibid.*, **34**, 528–39 (1951).
(9) Tschitschibabin, A. E., *J. Russ. Phys. Chem. Soc.*, **47**, 1582 (1915); *Chem. Zent.*, **1916, III**, 229.
(10) Tschitschibabin, A. E., *J. Russ. Phys. Chem. Soc.*, **50**, 513–16 (1918); *Chem. Zent.*, **1923, III**, 1022.

RECEIVED for review May 12, 1954. Accepted December 22, 1954.

Order of Adsorption Affinities of Polynitrostilbenes

ABNER F. TEAGUE[1], WILLIAM A. GEY, and ROBERT W. VAN DOLAH[2]
Chemistry Division, U. S. Naval Ordnance Test Station, Inyokern, China Lake, Calif.

The relative adsorption affinities of a series of polynitrostilbenes have been evaluated for two adsorbents. In the case of silicic acid, adsorbability varies in the expected manner, increasing with number of nitro groups. In the case of kaolin, the order is almost completely reversed, in contrast to the previously published and accepted reports.

IN CONNECTION with a study of the role of various compounds in retarding the crystallization of molten nitrotoluenes (*6*, *8*), it was postulated that the more effective additives were operating through adsorption processes for which isotherms could be developed. Such a mechanism had been proposed earlier by Freundlich (*7*) who found, for a number of systems, a correlation between adsorption on charcoal and the ability of various additive compounds to retard the linear crystallization velocity.

In the hope of obtaining similar corroborative evidence in the nitrotoluene systems, the relative adsorption affinities of a series of polynitrostilbenes for two adsorbents were evaluated by means of chromatographic column techniques.

EXPERIMENTAL

The nitrostilbenes were prepared in the usual manner by condensing 2,4,6-trinitrotoluene or 2,4-dinitrotoluene with the proper benzaldehyde in boiling xylene to which had been added a few drops of piperidine. By this reaction and subsequent recrystallizations, 2,4-dinitrostilbene (*15*), melting point 138–139° C.; 2,3′,4-trinitrostilbene (*15*), melting point 181–182° C.; 2,4,4′-trinitrostilbene (*11*), melting point 239.5–240° C.; 2,4,6-trinitrostilbene (*16*), melting point, 157–158° C.; 2,3′,4,6-tetranitrostilbene (*1*), melting point, 160–160.5° C.; 2,4,4′,6-tetranitrostilbene (*1*), melting point, 195–196° C.; and 2,2′,4,4′-tetranitrostilbene (*9*), melting point, 263° C. dec. (uncorrected) were prepared. The assumption was made, based on the method of preparation and characteristics of the compounds, that all were in the trans configuration (*4*).

Chromatographic tubes, 2 cm. in diameter and 20 cm. in length, were packed with adsorbent to a depth of 12 to 15 cm. Packing and chromatography were performed using the maximum reduced pressure obtained from a Welch Duo-Seal pump. The adsorbents used consisted of either a mixture of 3 parts (by weight) of silicic acid (Merck reagent grade) to 1 part Celite 535 (Johns-Manville Corp.), or a mixture of 5 parts of kaolin (J. T. Baker, washed and ignited N. F. grade) to 3 parts of Celite 535. Other adsorbents were tried but found to be less desirable. Thus, chromatograms on alumina did not develop properly and by virtue of color development gave indications of profound reactions taking place. Calcium carbonate mixed with Celite did not effect adequate adsorption, while pure calcium carbonate gave poor separations. Similarly, titanium dioxide gave only poor adsorption.

To determine the behavior of single compounds on the column and toward streak reagents, 10 mg. of the desired nitrostilbene were dissolved in 4 ml. of benzene and this solution was diluted with 9 ml. of Skellysolve B (Skelly Oil Co.). The column was wet with V ml. of Skellysolve B (where V ml. was the minimum volume of liquid to wet the packing completely). The nitrostilbene solutions were introduced onto the column just prior to the disappearance of the prewetting solvent. The chromatogram was developed by passing a solution consisting of 1 part (by volume) of benzene to 4 parts of Skellysolve B through the column. The development required between 1.5 V and 3 V ml. to obtain well-defined zones on the column. Development was completed by washing with 0.8 V ml. of Skellysolve A. Because of inadequate color development by the nitrostilbenes themselves, the column was extruded and examined by means of streak reagents. Ultraviolet light was not found to be useful in the identification of zones of adsorption.

The streak reagents were prepared as follows:

Vanadium pentoxide, 0.25% dissolved in 85% sulfuric acid.

Diphenylamine (*14*), 0.25% in C.P. sulfuric acid.

Ceric sulfate (*14*), 1.00 gram in 15 grams of distilled water diluted with 84 grams of C.P. sulfuric acid.

Sodium hydroxide (*14*), 6N solution.

Potassium dichromate (1%) and potassium nitrite (*14*) (1%), both in C.P. sulfuric acid, were found not to give adequate color tests.

Table I lists the color reactions found with the various nitrostilbenes when adsorbed on the two adsorbents.

For determining relative adsorption affinities, solutions of pairs of the nitrostilbenes were prepared as described above and their relative positions were determined. In these cases, the amount of solution used for development was determined by the minimum amount previously found necessary to obtain well-defined zones of the less strongly adsorbed compound. Sufficient pairs were taken in every case to ensure the proper ranking of all. Table II gives the results obtained.

DISCUSSION

As can be seen from the results shown in Table II, the original aims in conducting this work were hardly achieved, since a nearly complete reversal of order of adsorbability was obtained when the adsorbent was changed. The ability of the nitrostilbenes to retard the linear crystallization velocity of molten TNT fairly closely corresponds to the relative adsorption on silicic acid (*8*).

The relative adsorbabilities of many series of compounds have been reported; for summaries of the earlier work reference may be made to Zechmeister and Cholnoky (*17*) and Cassidy (*5*). In

[1] Present address, Bureau of Ordnance, Department of Navy, Washington, D. C.

[2] Present address, Explosives and Physical Sciences Division, Bureau of Mines, Pittsburgh, Pa.

Table I. Characterization by Streak Tests of Nitrostilbenes on Silicic Acid–Celite and Kaolin-Celite Adsorbents

Compound	Unstreaked Column	Streak Reagent: Ceric sulfate	Vanadium pentoxide	Sodium hydroxide	Diphenylamine	Diphenylamine over sodium hydroxide
Silicic Acid–Celite Adsorbent						
2,4,4′,6-Tetranitrostilbene	Pea green	Yellow tan	Light yellow	Brick red	...	...
2,3′,4,6-Tetranitrostilbene	Light tan	Brown	Red brown	Bright red	Pea green	...
2,2′,4,4′-Tetranitrostilbene	Light yellow	Yellow tan	Light yellow	Red	Tan	...
2,4,6-Trinitrostilbene	Canary yellow	Red orange	Red orange	Brick red	Tan	Light tan
2,4,4′-Trinitrostilbene	Light yellow	Pea green	Pea green	Pink	Pea green	Pea green
2,3′,4-Trinitrostilbene	Pea green	Yellow orange	Yellow tan	[a]	Pea green	Light tan
2,4-Dinitrostilbene	Light yellow	Olive green	Light green	[a]	...	Olive green
Kaolin-Celite Adsorbent						
2,4,4′,6-Tetranitrostilbene	Pea green	Light tan	Tan	Pink	...	...
2,3′,4,6-Tetranitrostilbene	Pea green	Pea green	Yellow	Pink	Blue green	Light blue green
2,2′,4,4′-Tetranitrostilbene	Pea green	Light tan	Tan	Pink	Light tan	Light tan
2,4,6-Trinitrostilbene	Canary yellow	Olive green	Pea green	[b]	[a]	Light blue
2,4,4′-Trinitrostilbene	Pale yellow	Pea green	[a]	[a]	Light green	Pea green
2,3′,4-Trinitrostilbene	Pea green	Light tan	Pea green	[a]	Dark pea green	Blue green
2,4-Dinitrostilbene	Pea green	Light tan	Blue gray	[a]	Tan	Pea green

[a] Reagent had no effect on color of compound.
[b] Turns orange on standing.

many cases the reported results have been obtained in effecting the isolation and identification of pure compounds from their mixtures, or in the study of the adsorbabilities of homologous series of compounds. Few studies have been made on the effects of substitution on adsorbability. Brockman and Volpers (*3*), using adsorbents modified to make them fluorescent, found with *p*-substituted stilbenes, unsaturated ketones, and certain benzoate esters that adsorption affinity decreased in the order: carboxyl, hydroxyl, keto, and ester. These authors ascribed the tenacity of binding to a combination of skeletal influences and the number and kind of functional groups. Further, Brockman (*2*) reported that substituted azobenzenes exhibited the same order of adsorbability from benzene–petroleum ether solution on aluminum oxide, silicon oxide, magnesium oxide, calcium sulfate, and copper sulfate, but found some differences in orders when diethyl ether was employed as the solvent. This author anticipated that deviations from the expected sequence, based on the additive effects of skeleton and substituents, would be observed when the adsorption behavior of the functional groups was very similar. This is indeed the case in the work reported herein, where the compounds vary only in number and position of nitro groups. LeRosen attempted to obtain a precise relation between electronic properties of substituents in the ortho and para positions of aromatic compounds and *R* (the ratio of rate of movement of the adsorbed compound in the column to the movement of the developing solvent in the column) (*10*). He found, however, that the influence of such factors as steric hindrance, inductive effects, solvent effects, spatial arrangements, internal hydrogen bonding, electronegativity of secondary groups, etc., was too great to allow this simple evaluation.

Few chromatographic studies on nitro compounds are reported. Schroeder and coworkers (*12–14*), in their elegant study of the fate of stabilizers in double-base powder, made extensive use of chromatograms. They found, for nitro-substituted diphenylamines, the following adsorption affinity for silicic acid–Celite: 2,2′,4,4′,6,6′-hexanitro- >2,2′,4,4′,6-pentanitro- >4,4′-dinitro- >2,2′,4,4′-tetranitro- >2,4,4′-trinitro- >2,2′,4-trinitro- >4-nitro- >2,4′-dinitro- >2,4-dinitro- >2,2′-dinitro- >2-nitro- (*13*). In this series there is stronger adsorption with the larger number of nitro groups except that 4-substitution creates abnormally high adsorption affinity and 2-substitution, abnormally low. The authors ascribe the latter effect to chelation with the amino group.

In the case of the nitrostilbenes, there is strongest adsorption on silicic acid with the largest number of nitro groups. Symmetry in the case of 2,2′,4,4′- may be the reason for this compound being the most strongly adsorbed. Substitution in the 3′-position appears to enhance the adsorption over the corresponding 4′-compounds. The nearly complete reversal of affinities on kaolin is in sharp conflict with the concept of Brockman that adsorbability depends on the number and kind of substituents.

Table II. Relative Adsorption Affinities of Various Stilbenes for Silicic Acid–Celite and Kaolin-Celite Adsorbents

Degree of Adsorption	Adsorbent: Silicic acid–Celite 535	Kaolin-Celite 535
Greatest	2,2′,4,4′-Tetranitrostilbene	2,4-Dinitrostilbene
	2,3′,4,6-Tetranitrostilbene	2,4,4′-Trinitrostilbene
	2,4,4′,6-Tetranitrostilbene	2,3′,4-Trinitrostilbene
	2,4,6-Trinitrostilbene	2,4,6-Trinitrostilbene
	2,3′,4-Trinitrostilbene	2,4,4′,6-Tetranitrostilbene
	2,4,4′-Trinitrostilbene	2,2′,4,4′-Tetranitrostilbene
Least	2,4-Dinitrostilbene	2,3′,4,6-Tetranitrostilbene

No definite arguments can be offered for this deviation in adsorption because of inadequate knowledge of the exact mechanisms involved in such processes. One is tempted to observe that the mechanisms must be fundamentally different in the two cases, quite in opposition to the generalization elaborated by Brockman. His finding of unexplainable variations induced by changes in solvent systems is corroboration of the concept that adsorption processes depend on the complete system, adsorbent, adsorbate, solvent, and extraneous materials, and generalizations should be approached with caution.

LITERATURE CITED

(1) Bishop, G., and Brady, O., *J. Chem. Soc.*, **1922**, 2364.
(2) Brockman, H., *Discussions Faraday Soc.*, No. 7, 58–64 (1949).
(3) Brockman, H., and Volpers, F., *Chem. Ber.*, **82**, 95–104 (1949).
(4) Calvin, M., and Alter, H. W., *J. Chem. Phys.*, **19**, 765 (1951).
(5) Cassidy, H. G., "Adsorption and Chromatography," Interscience, New York, 1951.
(6) Dalbey, E. R., and Gey, W. A., *J. Am. Chem. Soc.*, to be published.
(7) Freundlich, H., "Colloid and Capillary Chemistry," Methuen, London, 1926.
(8) Gey, W. A., Dalbey, E. R., and Van Dolah, R. W., *J. Am. Chem. Soc.*, to be published.
(9) Green, A., and Boddiley, J., *J. Chem. Soc.*, **1908**, 1721.
(10) LeRosen, A. L., Carlton, J. K., and Moseley, P. B., ANAL. CHEM., **25**, 666 (1953).
(11) Nisbet, H. B., *J. Chem. Soc.*, **1927**, 2081.
(12) Schroeder, W. A., Keilim, B., and Lemmon, R. M., *Ind. Eng. Chem.*, **43**, 939–46 (1951).
(13) Schroeder, W. A., Malmberg, E. W., Fong, L. L., Trueblood, K. N., Landerl, J. D., and Hoerger, E., *Ibid.*, **41**, 2818–17 (1949).
(14) Schroeder, W. A., Wilson, M. K., Green, C., Wilcox, P. E., Mills, R. S., and Trueblood, K. N., *Ibid.*, **42**, 539–46 (1950).
(15) Thiele, E., *Ber.*, **34**, 2843 (1901).
(16) Ullmann, F., and Geschwind, M., *Ibid.*, **41**, 2291 (1908).
(17) Zechmeister, L., and Cholnoky, L., "Principles and Practice of Chromatography," Wiley, New York, 1941.

RECEIVED for review October 25, 1954. Accepted January 3, 1955.

Detection of Surface-Active Agents Containing Polyoxyethylene or Polyoxypropylene Group

By Pyrolysis with Phosphoric Acid

MILTON J. ROSEN

Department of Chemistry, Brooklyn College, Brooklyn, N. Y.

Although a number of qualitative tests for the polyoxyethylene group have recently appeared in the literature, they suffer from a lack of specificity. The present test depends upon the thermal decomposition of the polyoxyethylene linkage in the presence of phosphoric acid to yield acetaldehyde, which produces a blue color with sodium nitroprusside and diethanolamine. The polyoxypropylene group, under the same conditions, yields propionaldehyde and its polymers, which produce orange colors. Positive results are obtained in the presence of the ester, alkylaryl, sulfide, sulfonate, sulfate, amino, amido, and phosphate groups. The only compounds which give positive results in the absence of the polyoxyethylene or polyoxypropylene groups are glycerides which, under the conditions of the test, decompose to acrolein, which also gives a blue color with sodium nitroprusside and diethanolamine.

As a result of the prevalent use of the polyoxyethylene group as a hydrophilic linkage in many of the newer surface-active agents, a number of qualitative tests for this functional group have recently appeared in the literature (*2–4, 9*). All of these tests, however, are based upon either the precipitation of the oxonium salt of the polyoxyethylene compound by a large anion, such as I_3^-, cobaltothiocyanate, or phosphomolybdate ion, or the appearance of turbidity upon heating an aqueous solution of the surfactant, due to the inverse temperature-solubility relationship of certain polyoxyethylene compounds.

The former type of test suffers from the fact that it is essentially a modification of the generally used precipitation test for cationic surfactants by means of a large anion (*1, 6, 9*) and therefore is given not only by polyoxyethylene compounds, but by all cationic surfactants, with or without the polyoxyethylene group in the molecule. On the other hand, the latter type of test is applicable only to water-soluble compounds and gives negative results with sulfonated polyoxyethylene compounds and the polyoxyethylene glycols, among others (*9*).

In a continuation of the search for simple, definitive tests for the functional groups present in commonly used surface-active agents (*5*), it has been found that all types of compounds containing the polyoxyethylene group may be detected very simply by pyrolyzing them in 85% phosphoric acid and leading the volatile products into an aqueous solution of sodium nitroprusside containing a water-soluble secondary amine, such as diethanolamine. The polyoxyethylene group, under these conditions, decomposes to yield acetaldehyde

$$-(-OCH_2CH_2-)_x \xrightarrow[\Delta]{H^+} nCH_3CHO + -(-OCH_2CH_2-)_{x-n}$$

which produces a blue color with the sodium nitroprusside and the secondary amine. This latter reaction is a reversal of the Simon test (*7*), in which secondary amines are detected by the blue color (of unknown structure) which they produce when treated with sodium nitroprusside and acetaldehyde.

In addition to detecting the polyoxyethylene group, this test can also be used to detect the polyoxypropylene group, which recently has been used in a number of surfactants to confer hydrophobic character to the molecule. Here, the polyoxypropylene group decomposes under the conditions of the test to yield propionaldehyde (which can be isolated as the 2,4-dinitrophenylhydrazone from the water-soluble fraction of the pyrolysis products) and its polymers, which produce orange colors with sodium nitroprusside and diethanolamine.

$$-(-OCH_2\underset{\displaystyle CH_3}{\underset{|}{CH}}-)_x \xrightarrow[\Delta]{H^+} n\ CH_3CH_2CHO + (CH_3CH_2CHO)_m + -(-OCH_2\underset{\displaystyle CH_3}{\underset{|}{CH}}-)_{x-(n+m)}$$

PROCEDURE

Place 200 mg. (or 4 drops) of anhydrous surfactant and 1 to 1.5 ml. of 85% phosphoric acid in a 5- or 6-inch test tube and agitate thoroughly for a few seconds. Insert a 0.5-inch plug of absorbent cotton into the mouth of the test tube (to prevent condensed water vapor from falling back into the hot reaction mixture with consequent violent spattering during the pyrolysis) and attach a glass delivery tube with a 60° angle bend by means of a one-hole rubber stopper. Clamp the test tube at an angle of about 30° from the horizontal, so that the main portion of the delivery tube is vertical. The end of the delivery tube should pass beneath the surface of the "detecting solution" contained in a 4-inch test tube placed on a white surface to facilitate observation of any color change. The detecting solution consists of 1 ml. of water to which have been added 2 drops of sodium nitroprusside solution (20 grams of sodium nitroprusside dihydrate dissolved in 50 ml. of water and diluted with 450 ml. of methanol) and 1 drop of diethanolamine.

Heat the mixture of phosphoric acid and surfactant with a 2-inch flame until the mixture turns dark brown. If foaming is excessive, discontinue heating momentarily to allow foaming to subside somewhat, then heat the entire test tube surface strongly just above the level of the foam and then move the flame down to the upper level of the foam. As the foam disappears under the intense heating, move the flame down the tube, keeping it always at the upper level of the foam until the liquid portion of the mixture is once again being heated by the flame. Continue the pyrolysis until either a blue color or an orange color (which may or may not change to dark brown) appears in the detecting solution (positive result) or for a maximum of 5 minutes if no blue or orange color appears (negative result).

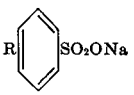
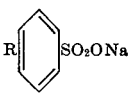
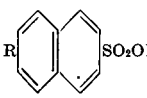
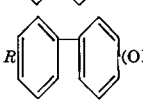
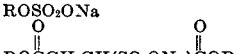
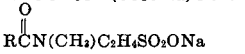
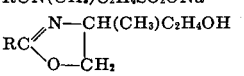
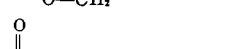
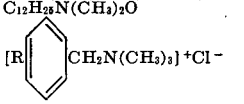
DISCUSSION OF RESULTS

Every type of surfactant tested which contains either the polyoxyethylene or the polyoxypropylene group, or both, gives a positive result with this test. This includes anionics (Triton X-200, Alipal CO-436), cationics (Ethomeens, Priminoxes, Hyamine 10-X), and nonionics (Igepals, Ethomids, Tweens, Pluronics, Victawet 12), containing such other functional groups as the ester linkage (Emulphor VN-430, Sterox CD), the alkylaryl group (Igepals, Triton X-100), the sulfide linkage (Sterox SE and SK), the sulfonate group (Triton X-200), the sulfate group (Alipal CO-436), the amino group (Ethomeens, Priminoxes), the amide linkage (Ethomids), and the phosphate group (Victawet 12). Surfactants containing only the polyoxyethylene group (Table I) produce a blue color; those containing only the polyoxypropylene group (Table II) produce an orange color; those containing both the polyoxyethylene and the polyoxypropylene groups (Table II) produce an orange color which quickly turns dark brown.

Table I. Reactions to Pyrolysis in 85% Phosphoric Acid of Surfactants with and without Polyoxyethylene Groups

Product	Source	Structure[a]	Color Produced	Result
Emulphor VN-430	Antara	$H(OC_2H_4)_xO\overset{O}{\overset{\|}{C}}R$ (oleic acid ester)	Royal blue	+
Sterox CD	Monsanto	$H(OC_2H_4)_xO\overset{O}{\overset{\|}{C}}R$ (tall oil ester)	Royal blue	+
Diglycol oleate L	Kessler	$H(OC_2H_4)_2O\overset{O}{\overset{\|}{C}}R$ (oleic acid ester)	Royal blue	+
Tergitol TMN[b]	Carbide & Carbon	$H(OC_2H_4)_xOR$ (trimethylnonyl ether)	Royal blue	+
Emulphor ON-870	Antara	$H(OC_2H_4)_xOR$ (oleyl ether)	Royal blue	+
Igepal CO-530	Antara	$H(OC_2H_4)_xO$–C$_6$H$_4$–R	Royal blue	+
Igepal CA-710	Antara	$H(OC_2H_4)_xO$–C$_6$H$_4$–R	Royal blue	+
Triton X-100	Rohm & Haas	$H(OC_2H_4)_xO$–C$_6$H$_4$–R	Royal blue	+
Sterox SE	Monsanto	$H(OC_2H_4)_xSR$	Red-purple then blue[c]	+
Sterox SK	Monsanto	$H(OC_2H_4)_xSR$	Red-purple, then blue[c]	+
Tween 40	Atlas	Polyoxyethylene sorbitan mono-palmitate	Royal blue	+
Tween 80	Atlas	Polyoxyethylene sorbitan mono-oleate	Royal blue	+
Triton X-200[b]	Rohm & Haas	R–C$_6$H$_4$–$(OC_2H_4)_2SO_2ONa$	Royal blue	+
Alipal CO-436[b]	Antara	R–C$_6$H$_4$–$(OC_2H_4)_xOSO_2ONH_4$	Royal blue	+
Ethomeen C/15	Armour	$H(OC_2H_4)_x$, $H(OC_2H_4)_y$ > NR ($x + y = 5$)	Royal blue	+
Ethomeen 18/60	Armour	$H(OC_2H_4)_x$, $H(OC_2H_4)_y$ > NR ($x + y = 50$)	Royal blue	+
Priminox 32	Rohm & Haas	$H(OC_2H_4)_{25}NHR$	Royal blue	+
Priminox 43	Rohm & Haas	HOC_2H_4NHR	Royal blue	+
Ethomid C/15	Armour	$H(OC_2H_4)_x$, $H(OC_2H_4)_y$ > $N\overset{O}{\overset{\|}{C}}R$ ($x + y = 5$)	Royal blue	+
Ethomid HT/60	Armour	$H(OC_2H_4)_x$, $H(OC_2H_4)_y$ > $N\overset{O}{\overset{\|}{C}}R$ ($x + y = 50$)	Royal blue	+
Hyamine 10-X	Rohm & Haas	[R–C$_6$H$_4$–$(OC_2H_4)_2N(CH_3)_2CH_2$–C$_6$H$_4$–] $^+Cl^-$	Royal blue	+
Hyamine 1622	Rohm & Haas	[R–C$_6$H$_4$–$(OC_2H_4)_2N(CH_3)_2CH_2$–C$_6$H$_4$–] $^+Cl^-$	Royal blue	+
Victawet 12	Victor	$H(OC_2H_4)_xO\overset{O}{\overset{\|}{P}}(OR)_2$	Royal blue	+
Ninol HA10	Ninol	1:1 Coc.F.A.-diethanolamine condensate	Royal blue	+
Ninol 201	Ninol	1:2 oleic acid-diethanolamine condensate	Royal blue	+
Glyceryl monostearate S	Glyco	$HOCH_2CHOHCH_2O\overset{O}{\overset{\|}{C}}R$	Royal blue	+
Arctic Syntex M	Colgate	$NH_4OSO_2OCH_2CHOHCH_2O\overset{O}{\overset{\|}{C}}R$	Royal blue	+
Castor oil	...	Triglyceride	Royal blue	+
Cottonseed oil	...	Triglyceride	Royal blue	+
Phosphated castor oil	Victor	.....	Royal blue	+
Span 40	Atlas	Sorbitan monopalmitate	Yellow	–
Span 80	Atlas	Sorbitan mono-oleate	Yellow	–
Arlacel C	Atlas	Sorbitan sesquioleate	Yellow	–
Santomerse D	Monsanto	R–C$_6$H$_4$–SO_2ONa	Pale pink	–

Table I. Reactions to Pyrolysis in 85% Phosphoric Acid of Surfactants with and without Polyoxyethylene Group (*Continued*)

Product	Source	Structure[a]	Color Produced	Result[b]
Alkanol WXN[b]	Du Pont		Beige	–
Ultrawet SK[b]	Atlantic		Cloudy white	–
Alkanol B	Du Pont		Beige	–
Areskap 100	Monsanto		Beige	–
Duponol ME	Du Pont	$ROSO_2ONa$	Beige	–
Aerosol OT	Amer. Cyan.		Beige	–
Igepon T-73	Antara		Light amber	–
Alkaterge-C	Coml. Solvents		Yellow	–
Glucaterge-28[b]	Coml. Solvents		Pale yellow	–
Ammonyx AO[b]	Onyx Oil	$C_{12}H_{25}N(CH_3)_2O$	Pale yellow	–
Hyamine 2389[b]	Rohm & Haas		Pale yellow	–

[a] R = alkyl or alkenyl group.
[b] Dried at 115° C.
[c] Initial red-purple color may be due to volatile sulfides produced in pyrolysis.

Table II. Reactions of Surfactants Containing the Oxypropylene or Polyoxypropylene Group to Pyrolysis in 85% Phosphoric Acid

Product	Source	Structure	Color Produced	Result
G-917	Atlas	Propylene glycol monolaurate	Orange	+
G-923	Atlas	Propylene glycol mono-oleate	Orange	+
G-2800	Atlas	Polyoxypropylene mannitol dioleate	Orange	+
Onyxol 368	Onyx Oil	Lauric isopropanolamide	Orange	+
Pluronic F68	Wyandotte	Propylene oxide–ethylene oxide polymer	Orange, then brown	+
Pluronic L62	Wyandotte	Propylene oxide–ethylene oxide polymer	Orange, then brown	+
Tergitol XC	Carbide & Carbon	Alkyl ether of propylene oxide–ethylene oxide polymer	Orange, then brown	+

The absence of a blue color in the case of surfactants containing both the polyoxyethylene and the polyoxypropylene groups is presumably due to prior decomposition of the polyoxypropylene group, producing the observed initial orange color. Upon subsequent decomposition of the polyoxyethylene group, instead of a blue color, dark brown is observed as a result of the combination of the initial orange color with the blue from the decomposing polyoxyethylene group.

The test is not limited to surface-active agents containing these functional groups, but is of application to derivatives of ethylene oxide or propylene oxide in general with the exception of ethylene glycol and propylene glycol. The results obtained with these derivatives are given in Table III.

The only type of compound tested which gives a blue color without containing the polyoxyethylene group is glycerides. This is due to the fact that glycerides, upon pyrolysis in acid medium, yield acrolein, which, when treated with sodium nitroprusside and secondary amines, produces a blue color similar to that produced by acetaldehyde (*8*). All other types of commonly used surfactants give negative results in the

Table III. Reactions of Derivatives of Ethylene Oxide and Propylene Oxide to Pyrolysis in 85% Phosphoric Acid

Product	Source	Structure	Color Produced
Ethylene glycol	Carbide & Carbon	$H(OCH_2CH_2)_xOH$ (x = 1)	Purple
Diethylene glycol	Carbide & Carbon	$H(OCH_2CH_2)_xOH$ (x = 2)	Blue
Polyethylene glycol 400	Carbide & Carbon	$H(OCH_2CH_2)_xOH$ (x ≅ 9)	Blue
Carbowax 4000	Carbide & Carbon	$H(OCH_2CH_2)_xOH$ (x ≅ 90)	Blue
Cellosolve	Carbide & Carbon	$HOCH_2CH_2OCH_3$	Blue
Tetraethylene glycol dimethyl ether	Ansul	$CH_3(OCH_2CH_2)_4OCH_3$	Blue
Propylene glycol	Carbide & Carbon	$H(OCHCH_2)_xOH$ (x = 1), CH_3	Pale yellow
Dipropylene glycol	Dow	$H(OCHCH_2)_xOH$ (x = 2), CH_3	Orange
Polyglycol P-400	Dow	$H(OCHCH_2)_xOH$ (x ≅ 7), CH_3	Orange
Polyglycol P-750	Dow	$H(OCHCH_2)_xOH$ (x ≅ 13), CH_3	Orange
Dowanol 50-B	Dow	$CH_3(OCHCH_2)_2OH$, CH_3	Orange
Monoethanolamine	Carbide & Carbon	$HOCH_2CH_2NH_2$	Blue
Diethanolamine	Carbide & Carbon	$(HOCH_2CH_2)_2NH$	Blue
Triethanolamine	Carbide & Carbon	$(HOCH_2CH_2)_3N$	Blue
Isopropanolamine	Carbide & Carbon	$HOCHCH_2NH_2$, CH_3	Orange
Diisopropanolamine	Carbide & Carbon	$(HOCHCH_2)_2NH$, CH_3	Orange
Triisopropanolamine	Carbide & Carbon	$(HOCHCH_2)_3N$, CH_3	Orange

absence of polyoxyethylene or polyoxypropylene groups (Table I).

LITERATURE CITED

(1) Flanagan, T. L., Jr., Drennen, T. J., and Goetchius, G. R., *Soap Sanit. Chemicals*, 24, No. 4, 163 (1948).
(2) Gnamm, H., "Die Lösungs und Weichmachungsmittel," p. 330, Stuttgart, 1941.
(3) Haakh, H., v. Candie, D., and Mobus, W., *Melliand Textilber.*, 32, 699 (1951).
(4) Newburger, S. H., *J. Assoc. Offic. Agr. Chemists*, 34, 109 (1951).
(5) Rosen, M. J., ANAL. CHEM., 27, 111 (1955).
(6) Shiraeff, D. A., *Chem. Specialties Mfrs. Assoc. Proc.*, 1950.
(7) Simon, L., *Compt. rend.*, 125, 536 (1897).
(8) *Ibid.*, p. 1105.
(9) Wurzschmitt, B., *Z. anal. Chem.*, 130, 105 (1950).

RECEIVED for review October 25, 1954. Accepted December 20, 1954. Presented before the Division of Polymer Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York.

Identification of Petroleum Refinery Wastes in Surface Waters

A. A. ROSEN and F. M. MIDDLETON

Robert A. Taft Sanitary Engineering Center, U. S. Public Health Service, Cincinnati, Ohio

Because the most significant pollution effect of petroleum refinery wastes is the production of tastes and odors in receiving waters, there is a need for nonsubjective methods of identification with sensitivity comparable to odor judgments. The aliphatic and aromatic hydrocarbon fractions separated from the wastes of five refineries showed corresponding infrared spectral patterns sufficiently characteristic to suggest their use in identification. The organic materials in three samples of surface waters containing varying amounts of refinery wastes were concentrated with active carbon. Hydrocarbon fractions, separated by chemical and chromatographic procedures, were identified by the resemblance of their infrared spectra to the spectral patterns of refinery waste hydrocarbons. This method provides chemical evidence, independent of odor evaluations, of the presence of low concentrations of petroleum refinery wastes in surface waters.

BECAUSE of their large volumes and high odor intensities (*6, 7, 17*), the most significant pollution effect of petroleum refinery process water effluents is the production of tastes and odors in receiving waters (*1, 5, 6, 15*). Indicative of the nature of this problem is the active current research on pollution abatement and the development of necessary analytical procedures (*8*). The major organic component of petroleum refinery wastes is the neutral group, consisting of hydrocarbons and closely related compounds which do not form salts with acids or bases. A large proportion of the odorous organic components is contained in the neutral group (*17*). Such compounds are resistant to biological and chemical action (*1, 5*); consequently, the odor effects persist beyond the immediate vicinity of the waste discharge.

Even in instances where the petroleum odor in water is recognizable, it is seldom possible by nonsubjective methods to establish the presence of petroleum products. Gross pollution has been detected by the presence of an oil film on the incoming water basins (*1*) and by paper chromatography of oil recovered from harbor slicks (*9, 18*). Melpolder, Warfield, and Headington (*13*) have recently published a very sensitive procedure for the identification and determination of volatile hydrocarbons in water. The method is limited to hydrocarbons boiling up to 400° F. and requires the use of a mass spectrometer. Descriptions have been published (*2, 17*) of the use in this laboratory of active carbon filters for the recovery and characterization of organic materials present in very low concentrations in surface waters. An application of these procedures (*14*) has been partially successful in demonstrating the presence of refinery wastes in water supplies drawn from a lake in the vicinity of the discharge points of a number of refineries. In this method, organic substances were recovered from the water by adsorption on activated carbon and subsequent elution, then were compared with a sample of refinery waste materials on the basis of elemental analysis, physical and chemical properties, and infrared spectra. The presence of oxygenated compounds, arising both from oxidation of the petroleum waste and from the presence of other types of pollutants, obscured the hydrocarbon properties serving as a basis for identification, particularly the infrared spectral characteristics. Petroleum hydrocarbons, consequently, were not clearly recognizable.

To minimize this interference, the method described in this paper utilizes adsorption chromatography on silica gel to remove oxygenated substances. In addition, the hydrocarbons are separated into an aliphatic and an aromatic fraction. Infrared spectra of corresponding hydrocarbon fractions of five refinery wastes showed a remarkable degree of similarity, suitable for use in identification. Similar infrared spectra were shown by the corresponding chromatographic fractions of organic compounds recovered from surface waters polluted with refinery wastes.

DEVELOPMENT OF METHOD

Chromatography. Adsorption chromatography is regularly applied to petroleum products, but its application to undistilled materials has been less frequent (*4, 10–12*). Wedgewood and Cooper (*19*) have detected polynuclear hydrocarbons in gas works waste by a combination of chromatography and ultraviolet spectroscopy. In developing the present procedure, preliminary experiments to evaluate adsorbents and operating conditions were performed. A sample containing equal weights of *n*-octadecane, 1-methylnaphthalene, and methyl stearate was chromatographed on five adsorbents: silica gel (Davison Codes 912, 923, and 950), alumina (Fisher A541/2), and carbon (Nuchar C-190 unground). Effluent portions of constant volume were collected separately, the quantity of residue in each fraction was estimated after evaporation of the solvent, and the composition of each residue was estimated from its infrared spectrum. Silica was the best adsorbent. The three silica gels were nearly equivalent in separation efficiency, but on the basis of flow characteristics, minimum discoloration, and economy, the Code 950 gel was slightly preferable. Separation was also improved by wetting the adsorbent with the first solvent before adsorption of the sample. Optimum volumes of eluting solvents were determined in similar experiments.

Recovery of Chromatographic Fractions. The quantitative recovery of petroleum materials from solution in volatile solvents is generally recognized to be a complex problem (*8*). The conditions finally adopted resulted in complete removal of solvent (as was shown by infrared spectra) with the attainment of con-

stant weight of high boiling residues, such as those in motor oil. Partial loss of volatile solutes, such as *n*-decane, could not be avoided.

PROCEDURE

The methods employed in obtaining samples of the organic components of surface waters and refinery wastes and of isolating the neutral fractions from the samples have been described (*2, 17*).

Silica gel (Davison Code 950) was tamped to a height of 10 cm. in a glass column 19 mm. in diameter. The weight of silica gel was 18 grams. The adsorbent was wetted with the first solvent, 2,2,4-trimethylpentane (iso-octane); the required volume was 17 ml. Five times this volume, 85 ml., of each solvent was used in the subsequent elutions. A sample not exceeding 1.5 grams of the neutral fraction of the carbon filter extract, dissolved in 4 ml. of iso-octane, was adsorbed on the top of the adsorbent column, then the remainder of the 85 ml. of solvent was percolated through the column. The effluent, containing the aliphatic hydrocarbon fraction, was collected in a tared flask. Similarly, the aromatic hydrocarbon fraction was eluted with benzene and the oxy (polar) fraction with a mixture of equal volumes of chloroform and methanol.

The neutral fraction of the organic substances obtained from some surface water samples contained a large proportion of material insoluble in iso-octane. In such instances, the sample was dissolved in benzene and chromatographed with the same solvent, yielding a fraction containing both aliphatic and aromatic hydrocarbons. This fraction was then further separated by the usual chromatographic procedure.

Each effluent fraction was concentrated to about 5 ml. by distillation or evaporation, then residual solvent was removed by evacuating to 30 to 50 mm. of mercury pressure at 50° C. for 1.5 hours. Eby (*3*) has published a similar procedure for the chromatographic separation of high boiling petroleum products into the same three kinds of fractions.

Infrared spectra of the solvent-free aliphatic and aromatic fractions were obtained by use of a Baird double-beam recording instrument with sodium chloride prism and windows, using a 0.10-mm. microcell at normal scanning speed (about 1 micron per minute). Infrared spectra of the oxy fractions were not useful for identification purposes.

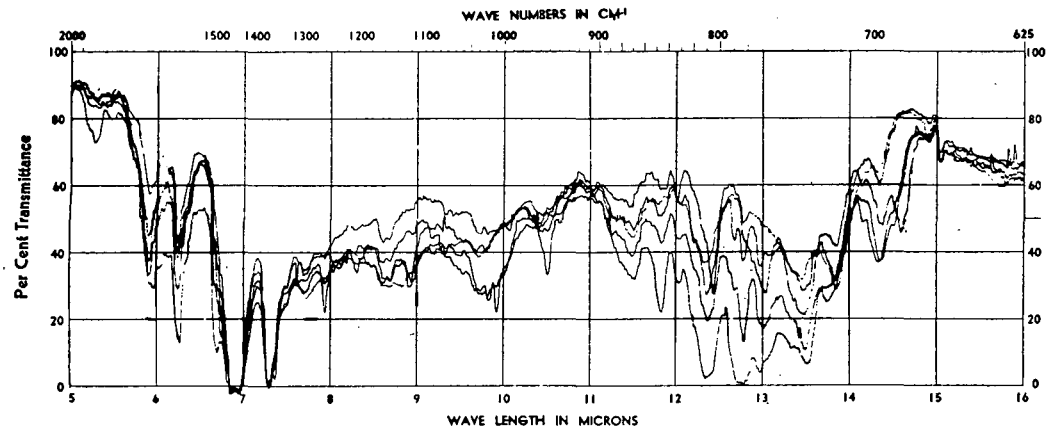


Figure 1. Refinery waste neutral fractions

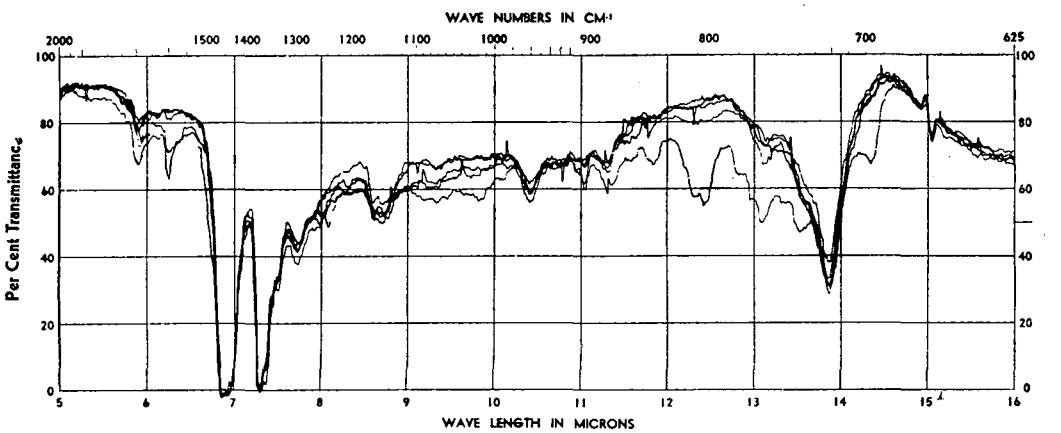


Figure 2. Aliphatic fractions of refinery wastes

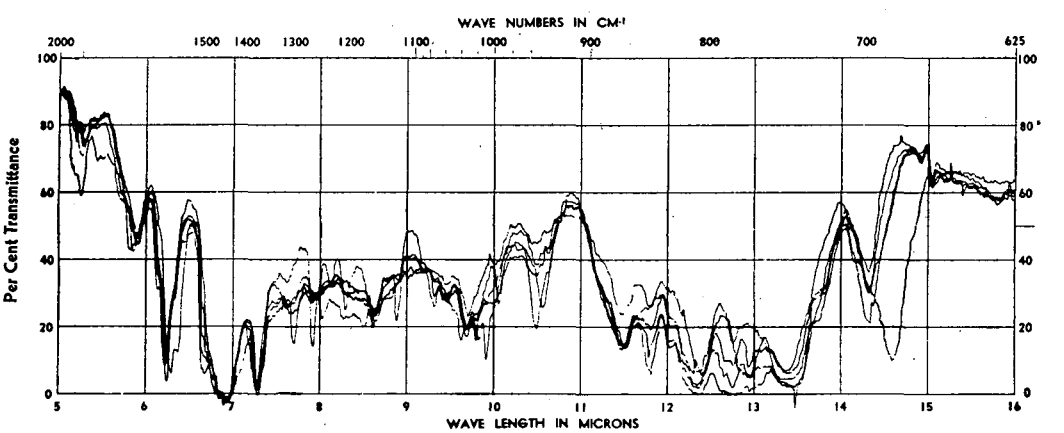


Figure 3. Aromatic fractions of refinery wastes

CHARACTERISTICS OF REFINERY WASTES

Samples of the organic materials in the waste effluents of five petroleum refineries were recovered by adsorption on activated carbon filters, in connection with a previous investigation of odor components. The samples described in this paper are identified as A-2, B-3, C-1, D-1, and E-1 in Table II of the earlier publica-

tion (*17*), which also describes in detail the sample collection procedure. Recoveries of organic materials ranged from 7.5 to 87 p.p.m. of the waste; the neutral fractions comprised 58 to 88% of the recovered material. Odor intensities of the total extracts and the neutral fractions showed little difference between refineries. The physical properties of the neutral fractions—i.e., density, refractive index, and boiling range—were too variable to serve as a means of identification.

Figure 1 depicts the infrared spectra of all the neutral fractions, recorded on the same chart and using the same cell and instrument gain setting. The results demonstrate a general pattern; however, in the "fingerprint" region between 9 and 15 microns the variations are sufficient to minimize the value of these spectra for recognition of refinery wastes. The infrared spectra of all the aliphatic hydrocarbon fractions obtained by chromatography of the neutral fractions are contained in Figure 2. It is evident that a characteristic pattern is presented by these spectra. A similarly characteristic pattern is revealed by the infrared spectra of the aromatic hydrocarbon fractions (Figure 3). Although the corresponding fractions of the wastes from the five refineries varied slightly in their spectra, these variations were with few exceptions in the strength rather than the position of the absorption bands, representing differing proportions of common components.

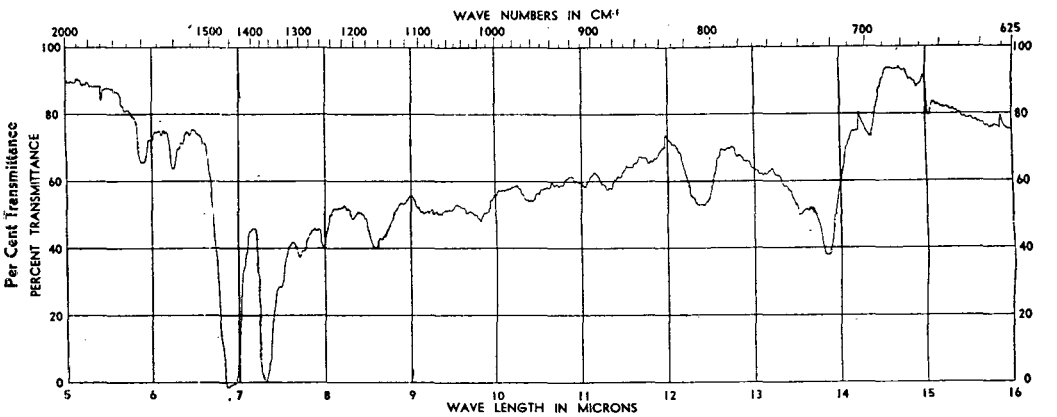


Figure 4. Aliphatic fraction from canal water

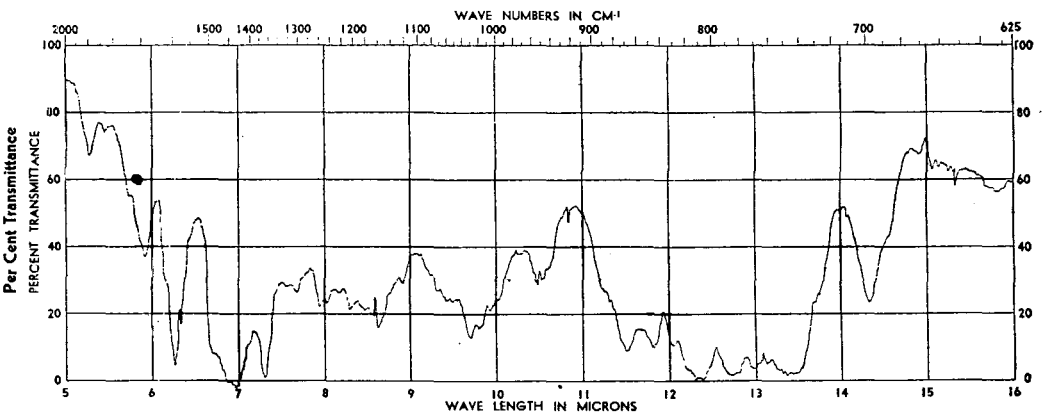


Figure 5. Aromatic fraction from canal water

The proportions of the three chromatographic fractions recovered from the wastes of the five refineries varied much more than did the infrared spectra of the fractions. The recoveries are presented in Table I. Losses encountered were primarily due to evaporation of more volatile components during removal of the eluting solvents. The characteristic petroleum odor was present principally in the aromatic fraction. The oxy fraction resembled asphalt in appearance and odor.

Sulfur was determined in the aliphatic and aromatic hydrocarbon fractions of the neutral components of wastes collected at three of the refineries. The results, shown in Table II, indicate that sulfur compounds, probably cyclic sulfides and substituted benzothiophenes (*16*), are regularly present in the chromatographed hydrocarbon fractions. Similar values for sulfur content have been reported for similar chromatographic fractions of crude oil (*11*). The values in Table II, however, appear to be too variable to offer a means of identification of refinery wastes.

APPLICATION OF METHOD

Canal Bordering Refinery Area. A total of 2480 gallons of canal water was pumped through a sand prefilter and then into a glass filter, 3 inches in diameter and 18 inches long, filled with unground active carbon, Nuchar C-190 (Industrial Chemical Sales Division, West Virginia Pulp and Paper Co., New York, N. Y.). The sand was discarded and the dried carbon was exhaustively extracted with chloroform, yielding 1.38 p.p.m. of a dark oil with characteristic petroleum odor, together with 0.06 p.p.m. of free sulfur in solid form. Group separation results were: 66% neutral compounds, 2% bases, 2% acids, 8% phenols, and 22% loss. The loss was attributed to compounds with unfavorable partition between ether and water. By chromatography, the neutral group separated into 33% aliphatics, 31% aromatics, and 13% oxy compounds. Figures 4 and 5 are the infrared spectra of the aliphatic and aromatic fractions. Figure 4 fits within the limits of Figure 2, and Figure 5 matches Figure 3 closely. The results are considered to confirm the presence of refinery wastes in the canal water.

Table I. Recovery of Chromatographic Fractions from Neutral Fraction

Refinery	Aliphatics, %	Aromatics, %	Oxy Compounds, %	Not Recovered, %
A	42	31	9	18
B	48	31	4	17
C	34	45	8	13
D	39	22	6	33
E	63	27	7	3

Table II. Sulfur Content of Refinery Waste Hydrocarbon Fractions

Refinery	Aliphatics, %	Aromatics, %
B	0.4	5.4
C	1.6	4.3
E	0.4	1.3

Lake Water Near Refinery Outfalls. A domestic water supply is drawn from a large lake at this point. Problems involving petroleumlike taste and odor in this supply have been encountered. Organic materials were recovered by pumping 20,160 gallons of raw water through a sand prefilter and then through a carbon filter consisting of a 3-foot length of iron pipe 4 inches in diameter, filled with 4–10 mesh Cliffchar granular active carbon (Cliffs Dow Chemical Co., Marquette, Mich.). The recovery of organic materials from the water amounted to 184 p.p.b., 45% of which was separated as the neutral fraction. Both the carbon filter extract and the neutral fraction prepared from it had strong refinerylike odors. Infrared spectral characteristics of these two samples resembled those of refinery wastes, but they were obscured by the presence of oxygenated compounds.

Chromatography of the neutral group yielded 42% aliphatics, 26% aromatics, and 18% oxy compounds. The infrared spectra of the aliphatic and aromatic fractions are shown in Figures 6 and 7. In this example, chromatography did not completely remove the oxygen compounds, as demonstrated by the 5.8-micron carbonyl band and the general absorption of the 8- to 10-micron region. In other respects the curves match closely the spectra of the corresponding fractions obtained from refinery wastes. These results are strong evidence for the presence of refinery waste materials in the lake water sample.

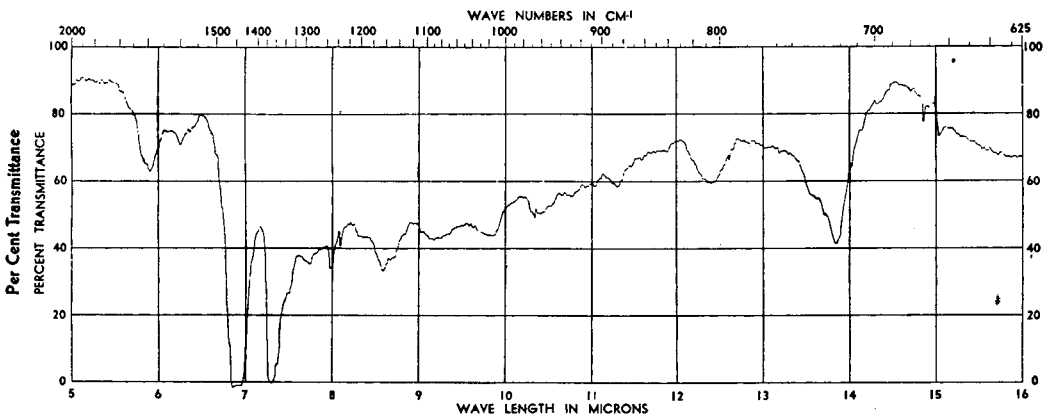


Figure 6. Aliphatic fraction from lake water

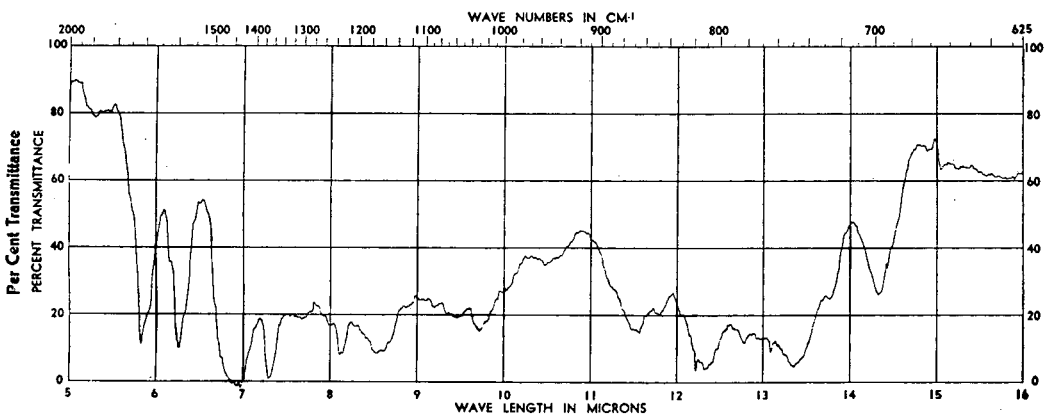


Figure 7. Aromatic fraction from lake water

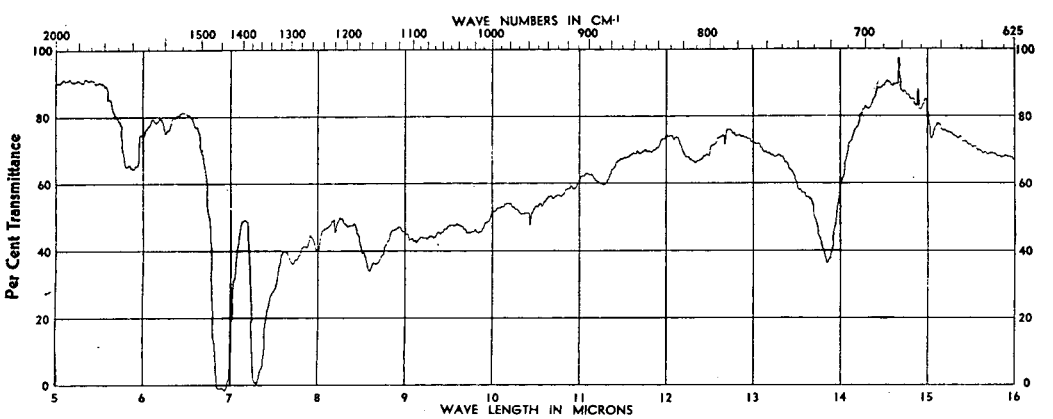


Figure 8. Lake water aliphatics, distant from refineries

Lake Water at a Distance from Refineries. Carbon filters were operated on both the raw and finished water supplies at a city located on the lake mentioned above, about 10 miles from the refineries. Water taste and odor problems at this city have been in part attributed to refinery wastes, this conclusion being based on the qualitative odor judgments of the water plant operators.

From 8120 gallons of the raw water, prefiltered with sand and then passed through an 18-inch-long filter containing 20-mesh active carbon, there was recovered 41 p.p.b. of organic material. At the same time, 9750 gallons of finished water passed through a similar carbon filter yielded 54 p.p.b. of organic extract. Each extract consisted of two layers: a lower brown wax and an upper light yellow oil. Infrared spectra of the oil layers suggested a mixture of hydrocarbons with oxygenated materials. The two extracts were combined for group separation, yielding 34% neutrals, 7% bases, 13% acids, 20% phenols (containing weak acids), and 1.5% aldehydes. The odors of the filter extracts and the neutral fraction did not resemble refinery wastes.

The neutral fraction was composed of two layers resembling

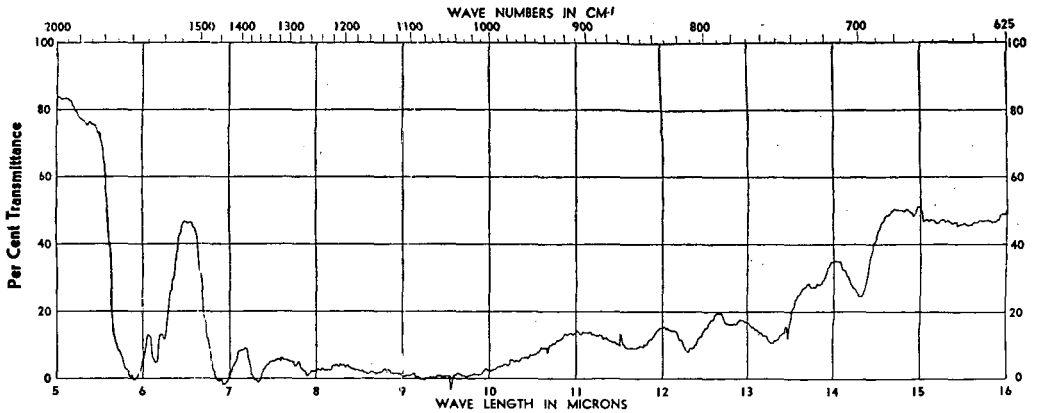


Figure 9. Lake water aromatics, distant from refineries

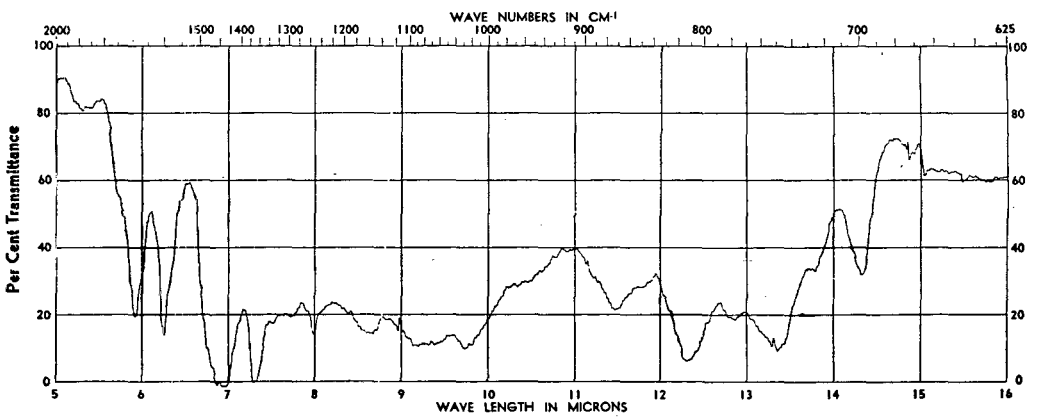


Figure 10. Aromatics distant from refineries, purified

those in the original filter extracts. Chromatography of this fraction yielded 32% aliphatics, 17% aromatics, and 51% oxy compounds. The infrared spectrum of the aliphatic fraction (Figure 8), though showing slight contamination by oxygen compounds, probably esters, still revealed the usual aliphatic hydrocarbon characteristics. The aromatic fraction could not be identified from its spectrum (Figure 9) as petroleum hydrocarbons because of its clearly high content of oxygen compounds.

As it appeared probable that the contamination was caused by esters, the aromatic fraction was saponified with alcoholic potassium hydroxide and nonsaponifiables were recovered by extraction with ether. The recovered extract, dissolved in benzene, was filtered through a 70 × 9 mm. column of silica gel (to remove alcohols formed in the saponification). The infrared spectrum of the recovered eluate (Figure 10), after removal of solvent, still exhibited slight characteristics of oxygen groupings. In other respects it closely matched the standard aromatic spectrum, Figure 3.

The spectra of the aliphatic fraction and the purified aromatic fraction thus support the conclusion that hydrocarbons resembling those in refinery effluents are present in the lake water at this sampling point. The hydrocarbons, however, constitute a minor portion of the organic substances recovered from the water.

DISCUSSION OF RESULTS

The method described above is probably less sensitive than a discriminating sense of odor in the detection of petroleum refinery wastes in water. The combination of operations employed, however, yields recordable information independent of the subjective nature of odor judgments. The present method is based on the properties of the hydrocarbon components. The characteristics of the hydrocarbons in other types of wastes, and consequently their possible interference in the method, have not yet been determined. Evidence provided by this method, therefore, offers presumptive, not conclusive, identification of water pollutants of petroleum origin.

Wastes containing hydrocarbon fractions spectrally distinguishable from those described here constitute a logical field for extension of this method.

ACKNOWLEDGMENT

The authors are grateful to Harry Braus and John Inskeep, who prepared some of the samples of organic materials from refinery wastes and surface waters. Microanalyses were performed by Paul W. K. Rothemund, Antioch College, and by Clark Microanalytical Laboratory, Urbana, Ill.

LITERATURE CITED

(1) Besozzi, L., and Vaughn, J. C., *J. Am. Water Works Assoc.*, **41**, 1035 (1949).
(2) Braus, Harry, Middleton, F. M., and Walton, Graham, ANAL. CHEM., **23**, 1160 (1951).
(3) Eby, L. T., *Ibid.*, **25**, 1057 (1953).
(4) Furby, N. W., *Ibid.*, **22**, 876 (1950).
(5) Gibbons, M. M., *Public Works*, **79**, 38 (1948).
(6) Giles, R. N., *Sewage and Ind. Wastes*, **24**, 1495 (1952).
(7) Giles, R. N., Scheineman, F. W., Nicholson, C. T., and Austin, R. J., *Ibid.*, **23**, 281 (1951).
(8) Headington, C. E., *et al.*, ANAL. CHEM., **25**, 1681 (1953).
(9) Herd, M., *Analyst*, **78**, 383 (1953).
(10) Hubbard, R. L., and Stanfield, K. E., ANAL. CHEM., **20**, 460 (1948).
(11) Karr, C., Weatherford, W. D., and Capell, R. G., *Ibid.*, **26**, 252 (1954).
(12) Lawrence, A. S. C., and Barby, D., *Discussions Faraday Soc.*, **7**, 255 (1949).
(13) Melpolder, F. W., Warfield, C. W., and Headington, C. E., ANAL. CHEM., **25**, 1453 (1953).
(14) Middleton, F. M., Braus, H., and Ruchhoft, C. C., unpublished data, Robert A. Taft Sanitary Engineering Center files, 1951.
(15) Muller, J. F., *Gas-u. Wasserfach*, **93**, 205 (1952).
(16) O'Donnell, Gordon, ANAL. CHEM., **23**, 894 (1951).
(17) Ruchhoft, C. C., Middleton, F. M., Braus, Harry, and Rosen, A. A., *Ind. Eng. Chem.*, **46**, 284 (1954).
(18) Schuldiner, J. A., ANAL. CHEM., **23**, 1676 (1951).
(19) Wedgewood, P. and Cooper, R. L., *Chemistry & Industry*, **1951**, 1066; *Analyst*, **78**, 170 (1953).

RECEIVED for review June 18, 1954. Accepted December 3, 1954. Presented before the Division of Water, Sewage, and Sanitation Chemistry at the 125th Meeting of the AMERICAN CHEMICAL SOCIETY, Kansas City, Mo., 1954.

Simultaneous Determination of Total Carbon and Carbon-14 Activity

Combustion Method

JOHN D. GABOUREL[1]

Division of Animal Husbandry, University of California, Davis, Calif.

MARY JANE BAKER and CHARLES W. KOCH

Department of Chemistry and Radiation Laboratory, University of California, Berkeley 4, Calif.

Standard wet-combustion procedures for the determination of total carbon are not adequate for many compounds. The standard Pregl combustion procedure is not satisfactory for determining activity of carbon-14–labeled compounds, because of radioactive cross contamination between consecutive samples. Data have been obtained using a combustion method in which the tube packing consists of platinum gauze, granular quartz, manganese dioxide spread on platinized asbestos, and silver wool. The method gives the total carbon content with an accuracy comparable to that obtained from the Pregl tube packing without encountering cross contamination in the subsequent determination of carbon-14 activity. The hydrogen content may be obtained from the same determination. The procedure should permit the simultaneous determination of total carbon and carbon-14 activity for any compound that can be analyzed by the conventional Pregl method.

AN ACCURATE carbon determination for use with carbon-14–labeled substances was provided by Buchanan and Nakao (*2*), but the apparatus is so complex that a simpler one is desirable for routine analytical purposes. Standard wet-combustion techniques such as the Van Slyke–Folch method and the periodate type digestion occasionally are not adequate for quantitative oxidation of the sample to carbon dioxide. For example, it has been found in this laboratory that sodium acetate, choline chloride, stilbamidine, and polystyrene yield low carbon analyses by the wet-combustion method, although accurate results are obtained using the conventional Pregl combustion procedure (*8*). The standard Pregl method would be ideal for burning a C^{14}-labeled sample to collect the carbon dioxide, except that radioactive cross contamination occurs between consecutive samples (*3*) because of the holdback of small amounts of carbon dioxide as well as of water on the packing in the combustion tube. Consequently, after the combustion of a carbon-14–containing sample, it is common practice to burn a substance which is inactive before burning a second carbon-14–labeled sample in order to eliminate this error caused by cross contamination. A tube packing different from the conventional one described by Pregl has been developed which does not retain any appreciable quantity of carbon dioxide and which gives an accuracy comparable to that of the standard method.

The principle is the same as that for the standard Pregl determination, in which a high temperature combustion in oxygen is employed to convert the carbon and hydrogen in the sample quantitatively to carbon dioxide and water. For measurements of carbon-14 activity, the carbon dioxide is absorbed in a standard sodium hydroxide solution. The amount of carbon dioxide then is determined by titration of the excess base. If a hydrogen analysis is required, the water is absorbed by Anhydrone, using a standard Pregl absorption tube (*9*). The carbon dioxide is collected either by passing the gas over Ascarite or by absorbing it in sodium hydroxide. The latter method always is employed when the carbon-14 activity is required.

Combustion to the desired products is made possible by passing the combustion gases over hot platinum gauze and granular quartz at 900° to 950° C. (see Figure 1). Interfering substances such as the halogens, sulfur, and phosphorus are removed by silver wool at 175° C. Any nitrogen dioxide which is formed during the combustion is removed by manganese dioxide. The use of manganese dioxide in place of lead dioxide has been described by Belcher and Ingram (*1*) and by Kirsten (*5*). In the present investigation the manganese dioxide was included in the combustion tube packing and was maintained at 175° C. in the thermostatic sleeve following the combustion furnace.

APPARATUS

The automatic semimicro carbon and hydrogen apparatus obtained from E. H. Sargent and Co. is suitable for this procedure. The furnaces tolerate continuous operation over the temperature range required. The pressure regulator and oxygen purification part of the combustion train are as described by Pregl (*8*). Standard combustion tubes made of quartz and of 96% silica glass may be used. If carbon-14 activity is to be determined or if the carbon is determined volumetrically, an absorber such as that shown in Figure 2 is used. The inner member is made from a fritted-glass disperser (Corning 39533-C). With the concentration of base used, this absorber removes carbon dioxide quantitatively from the gas stream even when oxygen flow rates are as great as 25 ml. per minute. The outer vessel (Fisher 3-038) has a capacity of 125 ml.

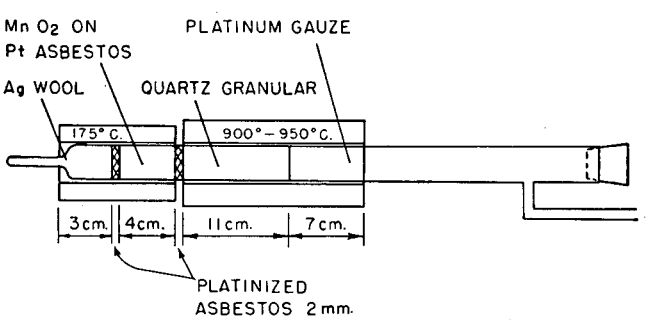


Figure 1. Combustion tube packing for carbon-hydrogen determination

For the gravimetric procedure, the conventional Mariotte bottle is used to control gas flow. A 20-liter Mariotte bottle is used to control the oxygen flow rate for the absorption of carbon dioxide by sodium hydroxide. A pressure head of approximately 4 feet of water is needed to overcome the back pressure of the fritted cylinder in the absorber.

PREPARATION OF TUBE PACKING

The materials used for the combustion tube packing require careful preparation. The granular quartz (30 to 50 mesh) must be cleaned with hot cleaning solution (concentrated sulfuric acid saturated with potassium or sodium dichromate), thoroughly washed with distilled water, and dried. The platinum gauze is cleaned with hot, concentrated nitric acid. Silver wool is used as obtained (Fisher Scientific Co., Chemical Catalog S-163);

[1] Present address, Chemical Corps Medical Laboratories, Army Chemical Center, Md.

platinized asbestos (5% platinum by weight) is used as obtained (J. T. Baker Chemical Co., Catalog 4-0922). It is important that the manganese dioxide be the purest available and it must be essentially free of alkaline earths. (Baker's analytical reagent grade manganese dioxide with a reported assay of 99.5 to 100.5% has proved satisfactory. The analysis listed for this preparation showed alkaline earths as sulfate present in the amount of 0.02%.) Manganese dioxide also is prepared by precipitation from permanganate in acid solution using enough hydrogen peroxide to reduce stoichiometrically the permanganate to manganese dioxide. The precipitate is thoroughly washed and is dried in vacuum at 100° C. for 15 hours.

REAGENTS FOR VOLUMETRIC METHOD

Standard hydrochloric acid, approximately 0.42*M*, was prepared from concentrated C.P. hydrochloric acid and standardized against primary standard sodium carbonate.

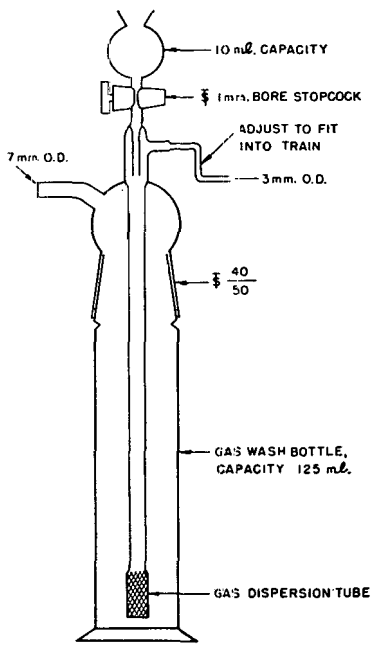


Figure 2. Carbon dioxide absorber

Standard sodium hydroxide, approximately 0.4*M*, was prepared from saturated sodium hydroxide. The bulk of the carbonate was removed from the saturated solution by filtration. This carbonate-free, saturated solution was then diluted to approximately 0.4*M* with carbon dioxide–free, distilled water and protected from further contamination by passing the incoming air over soda lime or Ascarite. This solution was standardized against standard hydrochloric acid prepared above under conditions which were used during actual analyses.

Barium chloride, 1.0*M*, was prepared from C.P. reagent and was protected from carbon dioxide.

Distilled water was boiled to remove carbon dioxide.

COMBUSTION PROCEDURE

To ensure complete combustion, the long furnace (see Figure 1) is operated at 900° to 950° C. The temperature of the thermostatic sleeve is 175° C. The movable furnace is operated at several temperatures in the course of the analysis. During the first burning the movable furnace is operated at 300° to 500° C. Flashing of the sample must be prevented to avoid incomplete combustion. Once the sample has been charred and there no longer is danger of flashing, the temperature is increased. For the first burning, the speed of the movable burner should not exceed 6 mm. per minute. For the second burning, which is the final one, the temperature of the movable furnace is increased to 850° C. and its speed is held to 16 mm. per minute. A gas sweeping time of at least 5 minutes is allowed after the final burning of the sample.

When activity measurements are made, the sample size should be sufficiently large to provide barium carbonate cakes of critical thickness. It is advisable to keep all barium carbonate cakes above critical thickness to avoid possible errors due to self-absorption. When a smaller sample is necessary, a critical weight curve must be obtained to correct for the varying effect of self-absorption. For the filtration apparatus used by the authors, the critical weight corresponds to 40 mg. of barium carbonate (apparatus obtainable from Tracerlab, Catalog No. E-88).

The oxygen flow rate is dependent on sample size. The standard flow rate of 4 to 5 ml. per minute is used for samples from 3 to 6 mg. For samples as large as 30 mg., the flow rate is increased to 20 to 25 ml. per minute. Approximately 40 to 45 minutes are required for each analysis.

VOLUMETRIC DETERMINATION OF CARBON

Ten milliliters of standard sodium hydroxide are pipetted into the absorbing vessel. Carbon dioxide–free distilled water is added until the absorber is approximately three fourths full. The absorber is stoppered with the inner member and placed in the combustion train. The 10-ml. bulb at the top of the absorber (see Figure 2) is filled with distilled water. This water is used to wash the inside of the disperser tube and the fritted cylinder after the combustion is completed. When the combustion is completed, the absorber is detached from the train. The bubbler is opened, the inner member is washed with distilled water, and the washings are collected in the outer vessel. Nitrogen gas or carbon dioxide–free air is bubbled through the solution. In addition to preventing absorption of carbon dioxide from the air, the bubbling gas aids in stirring the solution during titration. Three milliliters of 1.0*M* barium chloride (a two- to threefold excess) are added to the absorbing solution followed by 3 drops of 0.25% phenolphthalein indicator. The excess base in the resulting mixture is titrated with standard hydrochloric acid. A 10-ml. microburet graduated in 0.02-ml. divisions is used for the titration. Care must be taken to add the hydrochloric acid slowly in order to avoid local high concentration of hydrogen ion which will result in loss of carbon dioxide from the sample. After the titration is completed, the solution is made slightly alkaline (pink to phenolphthalein) for the preparation of a barium carbonate cake for counting. A Tracerlab SC-16 internal flow counter, employed as a Geiger-Müller counter, is used for activity determinations.

RESULTS

The combustion tube packing described herein was first tested using the volumetric method to determine carbon content. Typical results are listed in Table I.

After it was demonstrated that the tube packing gave reasonably reproducible carbon analyses, possible carbon-14 cross contamination was investigated. The fact that carbon-14 cross contamination did not occur is shown in Table II. The samples were analyzed in the order shown in the table.

Table I. Carbon Determinations Using Volumetric Method

Sample	Sample Wt., Mg.	C, % Theor.	C, % Found	Deviation, %
Benzoic acid	10.21	68.85	69.5	+1.0
	9.05		69.3	+0.7
	14.25		68.2	−1.0
Sucrose	18.51	42.1	42.1	0.0
	17.35		42.6	+1.1
	20.27		41.9	−0.5
	21.96		42.2	+0.3
	15.22		42.5	+1.0
Oxalic acid dihydrate	38.77	19.0	19.0	0.0
	45.05		18.9	−0.5

Table II. Barium Carbonate Activity Measurements Showing Absence of Cross Contamination[a]

Sample	$BaCO_3$ Activity, Net Counts/Minute
Inactive galactose	0.1
Randomly labeled lactose	11,732
Inactive galactose	0.6
Randomly labeled lactose	11,600
Inactive galactose	0.0
Randomly labeled lactose	11,700
Inactive galactose	0.2
Randomly labeled glucosazone	11,976
Inactive galactose	0.3
Randomly labeled glucosazone	12,010
Inactive galactose	0.4

[a] Samples analyzed in order shown.

The consistency of the results included in Table II is somewhat better than that generally found using solid counting methods. There is no apparent reason why this should be true and probably it is a consequence of a relatively limited number of measurements. Although the primary purpose in this investigation concerned the usefulness of the method for the determination of an accurate carbon and hydrogen as well as for obtaining the carbon-14 activity, it is unfortunate that the carbon-14 activity could not be determined by a gas counting method. However, at the time of these measurements, such equipment was not available for the use of the authors.

Table III. Carbon Determinations Using Gravimetric Method

Sample	Sample Wt., Mg.	C, % Theor.	C, % Found	Deviation, %
Benzoic acid	4.0–4.3	68.85	68.74	−0.2
			68.80	−0.1
Acetanilide	3.6–4.4	71.09	71.25	+0.2
			71.13	+0.1
			71.00	−0.1
Anthracene	3.0–3.8	94.34	93.86	−0.5
			94.07	−0.3
			94.19	−0.2
			93.94	−0.4
			93.74	−0.7
			93.75	−0.7
			94.29	−0.1
			94.16	−0.2
Fumaric acid	4.1–5.8	41.31	41.47	+0.4
			41.47	+0.4
			41.41	+0.2
Chlorobenzoic acid	3.5–5.0	53.67	53.97	+0.6
			53.80	+0.2
			53.50	−0.3
			53.88	+0.4
Iodobenzoic acid	5.2–7.3	22.31	22.55	+1.1
			23.04	+3.3
			22.41	+0.4
			22.53	+1.0

Table IV. Carbon and Hydrogen Determinations Using Gravimetric Method

Sample	Sample Wt., Mg.	C, % Theor.	C, % Found	Dev., %	H, % Theor.	H, % Found	Dev., %
Benzoic acid	3.5–5.1	68.85	69.11	+0.4	4.94	4.93	0
			68.98	+0.2		4.97	+1
			69.29	+0.7		5.01	+1
			69.33	+0.7		4.82	−2
			68.61	−0.4		4.78	−3
			69.07	+0.3		4.97	+1
			69.21	+0.5		4.83	−2
			69.05	+0.3		4.98	+1
			68.91	+0.1		5.03	+2
Acetanilide	3.4–4.6	71.09	71.33	+0.3	6.71	6.88	+3
			70.84	−0.4		6.82	+2
			70.98	−0.1		6.70	0
			71.20	+0.1		6.88	+3
			70.99	−0.1		6.68	0
Chloroacetanilide	3.6–5.4	56.65	56.48	+0.3	4.76	4.80	+1
			56.68	+0.1		4.72	−1
			56.55	−0.2		4.66	−2
			56.56	−0.2		4.86	+2

The reliability of the carbon determination using the quartz tube packing was tested by collecting the carbon dioxide and water in conventional Ascarite and Anhydrone absorption tubes. The flow rate was maintained at the standard rate of 4 to 5 ml. per minute. The results obtained for carbon are listed in Table III. The Anhydrone tube was used to remove water before absorption of the carbon dioxide on Ascarite, but the quantity of water produced was not determined. These results show that the carbon determinations obtained using this combustion tube packing are comparable to those obtained by the standard carbon-hydrogen procedure. Table IV lists determinations of both carbon and hydrogen using the quartz packing.

Finally, a series of experiments was carried out in which the hydrogen was determined gravimetrically and the volumetric method was used for carbon. A short Anhydrone absorber was placed between the absorption tube for water and the sodium hydroxide absorber as a precaution against back-diffusion of water vapor from the carbon dioxide collector. For these measurements in which 4- to 10-mg. samples were used, the carbon dioxide was collected in standard 0.1*M* base and the excess base was back-titrated with standard 0.1*M* acid. Two milliliters of 1.0*M* barium chloride were used to precipitate carbonate from the alkaline solution. The carbon dioxide collector was similar to the one shown in Figure 2 but the total volume of the bottle was approximately 30 ml. with a liquid height for 10 ml. of solution equal to 10 cm. The data are presented in Table V.

Table V. Carbon and Hydrogen Determinations Using Volumetric Carbon Procedure

Sample	Sample Wt., Mg.	C, % Theor.	C, % Found	Dev., %	H, % Theor.	H, % Found	Dev., %
Acetanilide	5.5–8.1	71.09	71.1	0.0	6.71	6.65	−1
			71.3	+0.3		6.76	+1
			71.2	+0.1		6.70	0
			71.5	+0.6		6.61	−2
Chloroacetanilide	5.8–8.4	56.65	56.4	−0.4	4.76	4.77	0
			56.7	+0.1		4.62	−3
			56.5	−0.3		4.80	+1
			56.7	+0.1		4.89	+3
Benzoic acid	4.5–5.2	68.85	68.8	−0.1	4.94	4.92	0
			68.7	−0.2		4.96	0
			68.9	+0.1		4.99	+1

For the data listed in Table V, flow rates approximating 5 ml. per minute were used. Greater rates of flow were not attempted. In addition to the data listed, it was found that no appreciable quantity of carbon-14 was held up by the Anhydrone in the course of several combustions in which inactive benzoic acid was analyzed after samples of carbon-14–labeled glucose having approximately 2000 counts per minute per mg.

SUMMARY

The combustion tube packing employed in this study eliminates cross contamination due to carbon-14 holdup. It affords a more reliable analysis for carbon than do wet digestion procedures and permits a simple acid-base titration for the analysis of carbon in organic samples which contribute other acidic gases on combustion. In addition, the combustion method has the advantage that from a single determination one can obtain a quantitative analysis for carbon and hydrogen with an accuracy comparable to the standard determination as well as carbon-14 activity.

The new tube packing has been used extensively by Kleiber and the Davis Tracer Team (*6*, *7*) for the combustion of carbon-14–labeled milk products and also by Gosselin, Gabourel, Kalser, and Wills (*4*) for tissue analysis of mice and rats for total carbon and carbon-14. This method has proved to be very satisfactory for routine analysis on biological materials.

ACKNOWLEDGMENTS

The authors wish to acknowledge the cooperation of Max Kleiber and the Davis Tracer Team, Division of Animal Husbandry, University of California, Davis, Calif. This work was supported in part by the U. S. Atomic Energy Commission.

LITERATURE CITED

(1) Belcher, R., and Ingram, G., *Anal. Chim. Acta*, 4, 124 (1950).
(2) Buchanan, D. L., and Nakao, A., *J. Am. Chem. Soc.*, 74, 2389 (1952).
(3) Calvin, Melvin, "Isotopic Carbon," pp. 82–8, John Wiley & Sons, New York, 1949.
(4) Gosselin, R., Gabourel, J., Kalser, S., and Wills, J., to be submitted to *J. Pharmacol. Exptl. Therap.*
(5) Kirsten, W., ANAL. CHEM., 25, 74 (1953).
(6) Kleiber, M., Div. of Animal Husbandry, University of California, Davis, Calif., unpublished.
(7) Kleiber, M., Black, A., Brown, M., Luick, J., Baxter, C., and Tolbert, B., *J. Biol. Chem.*, 210, 239 (1954).
(8) Pregl, F., and Grant, J., "Quantitative Organic Microanalysis," 5th ed., pp. 33–57, Blakiston, Philadelphia, 1951.
(9) *Ibid.*, pp. 44–6.

RECEIVED for review August 12, 1954. Accepted December 10, 1954.

Detection of Unsubstituted Para Position in Phenols

SAUL SOLOWAY and ANGELO SANTORO[1]

Department of Chemistry, The City College, College of the City of New York, New York, N. Y.

Phenols are readily oxidized to colored products in ammoniacal solution by persulfate ion in the presence of a catalytic amount of silver ion. Derivatives having a free para position are distinguished from others in yielding blue or green dyes, which are pH as well as redox indicators. Evidence is given for the belief that these dyes are derivatives of indophenol. The nature of the catalysis by silver ion and the anomalous behavior of a few phenols are discussed.

A DILUTE ammoniacal solution of phenol when oxidized by persulfate, hypochlorite, or hypobromite ions in the presence of a catalytic amount of silver ion was found by Escaïch (*1*) to yield a green or blue solution. The chemistry and the scope of this reaction have never been investigated.

In a review article on qualitative tests for the phenolic group Gibbs (*2*) suggested that the color developed in the Escaïch reaction was due to the oxidation of phenol by silver ammonium ion. The implication of this suggestion is that persulfate ion functions as the oxidant to maintain the concentration of the silver ammonium ion. A test of this hypothesis showed that Tollens reagent (silver ammonium ion) in quantity was incapable of producing the Escaïch color reaction. However, the addition of argentic oxide to an ammoniacal solution of phenol gave a green color. Since argentic oxide is prepared by the oxidation of argentous ion with alkaline persulfate, the nature of the silver ion catalysis in the Escaïch reaction is clear.

The structures of the green and blue compounds formed in this oxidation have not been elucidated. Gibbs (*2*) demonstrated that 2,6-dibromo-*N*-chloroquinoneimine condenses with a phenol having an open para position to produce the corresponding dibromoindophenol derivative. Singer and Stern (*4*) used the Gibbs test as the basis of a quantitative spectrophotometric determination of several phenols. Since phenol, ammonia, and an oxidant, the essential components of the Escaïch reaction, could produce an intermediate at the same oxidation level as *N*-chloroquinoneimine, the indophenol may possibly be the blue component produced in the reaction.

Experiments showed that of the many amines tested ammonia was specific for green or blue color formation. The colors produced with phenol in the reaction corresponded with the known colors of indophenol in both acid and alkali solutions. Further, the color could be bleached by reduction with zinc dust and reformed by autoxidation. This latter behavior is characteristic of indophenol dyes. A comparison of the absorption spectra of several reaction mixtures with that of a known solution of indophenol showed many similarities. However, they were far from identical, and the spectra of the reaction mixtures changed considerably with time. This observation was undoubtedly due to the fact that the colored intermediates were subjected to further oxidation with persulfate.

Several attempts were made at isolating and purifying the dye components by conventional methods. After several variations a chromatographic method yielded a solution which matched the spectrum of indophenol fairly well as shown in Figure 1. However, the amount of material recovered from the separation was too small to substantiate its identity by the preparation of derivatives.

The tests of 65 phenols showed that, with a few exceptions, only those derivatives which had unsubstituted para positions and did not contain strong electronegative groups such as nitro, cyano, carbonyl, amide and carbalkoxy, gave a blue or green color on oxidation with persulfate in ammoniacal solution containing silver ion. Other derivatives were either unoxidized or gave yellow or amber colors. Table I lists the results.

In order to obtain a positive result (blue or green color) with phenols containing electronegative substituents in ortho or meta positions, the compound was first boiled with zinc dust and alkali. This treatment either hydrolyzed or reduced these groups to electropositive ones which, if anything, enhanced the oxidation with persulfate.

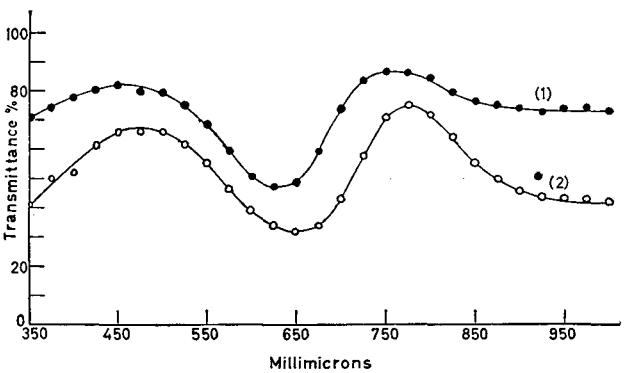


Figure 1. Absorption spectra

1. **Indophenol (purchased) in presence of potassium persulfate**
2. **Ether extract of chromatographed material**

A notable exception to the observation that a free para position was necessary for a positive test was the case of some para halogenated phenols. Qualitative tests showed the absence of chloride or oxidized ions of chlorine in these experiments. If it is assumed that the similar colors obtained were also the result of the formation of para indophenol derivatives, it follows that the halogen group migrated to another position on the ring.

EXPERIMENTAL

The addition of a few milligrams of solid potassium persulfate to an aqueous ammoniacal solution containing a catalytic amount of silver nitrate gave an excellent test with phenol and some of its low molecular weight derivatives, which were sufficiently soluble in the medium. However, with *m*-pentadecylphenol, the hydroxybiphenyls, and the hydroxystilbenes, it was necessary to add some aqueous alkali and/or pyridine to increase the solubility.

During the tests of a number of phenols, observations were that salicylanide, salicylonitrile, salicylate esters, *o*-hydroxyacetophenone, etc., gave yellow colors like the para substituted phenols. If these compounds were treated with zinc dust in alkaline solution prior to oxidation, they acted as typical phenols with free para positions.

The following procedure was therefore used to detect an unsubstituted para position in phenols. Ten to 20 mg. of a phenol was dissolved in 1 ml. of 2*M* aqueous sodium hydroxide. If the compound was not totally soluble, 2 or 3 drops of pyridine were added. Then 50 mg. of zinc dust was also added and the solution was refluxed for a maximum of 5 minutes. After centrifugation and decantation of the supernatant liquid, 2 ml. of concentrated ammonia, 2 drops of 2*M* silver nitrate, and 10 to 20 mg. of potas-

[1] Present address, Department of Chemistry, University of Kansas, Lawrence, Kan.

sium persulfate were added. Within 5 to 15 seconds a green, blue-green, or blue solution resulted for compounds containing open para positions. Otherwise, yellow, orange, or amber colors resulted. Regardless of the color the resulting solution was heated with 30 mg. of zinc dust. Centrifugation showed the supernatant liquid had become much lighter if not colorless. Decantation, followed by shaking with air, caused reoxidation to the previous color. In some cases a few drops of 3% hydrogen peroxide were added to accelerate the oxidation.

Several attempts were made at isolating the blue compound produced in the oxidation of 50-gram batches of phenol by the method described. In these experiments the phenol solution was not pretreated with zinc dust. The oxidation was carried out at 0° C. because the blue color was more stable at that temperature toward excess persulfate ion. After standing for about 1 hour the reaction mixture was poured into excess dilute hydrochloric acid and extracted with chloroform. The addition of ligroin to the chloroform extract caused a relatively small amount of a dark amorphous solid to precipitate. This material melted with decomposition at 85° to 90° C. Recrystallization from some common solvents proved ineffective in purifying this material. After several unsuccessful variations the following chromatographic procedure yielded a solution which gave an absorption spectrum almost identical with that of a known sample of indophenol.

A column of Merck's 100-mesh alumina, 10 inches high, was prepared by filling the absorption tube with ligroin (60° to 90° C. boiling range) followed by the addition of alumina in small amounts with vigorous shaking. After the alumina had settled, the ligroin was allowed to drain out until its surface coincided with that of the adsorbent. The sodium salt of the extract was dissolved in 0.5 ml. of absolute methanol and added to the column. The dye was strongly adsorbed at the top of the column. The column was then washed down with ligroin saturated with absolute methanol. While much of the material remained at the top of the column, some moved down to form another zone. This second zone was separated from the rest of the alumina column and extracted with ether. The ether was removed in vacuo, the residue dissolved in water, and the absorption measured as rapidly as possible with a Model B Beckman spectrophotometer. Figure 1 shows a comparison of the spectrum of this solution with that of a known solution of sodium indophenolate.

RESULTS AND DISCUSSION

The data of Table I show that for most of the derivatives tested, those with vacant positions para to the phenolic group gave green or blue colors. Certain anomalies, however, did manifest themselves. The orange color obtained in the case of 2-hydroxy-4-aminobenzoic acid is probably due to simultaneous oxidation of the amino group with the consequent formation of a competing chromophore. 8-Quinolinol also gave an orange color, typical of many phenols with electronegative groups. Unlike the nitro or carbonyl groups, which are readily reduced by zinc dust in alkaline solution, the pyridine ring in this case remains intact.

The observation of color formation in two stages proved repetitious in most cases, but was helpful in the case of 2,4-dihydroxybenzoic acid. The latter gave an orange-red color in the first stage and a blue-green in the second. This behavior may be due to the presence of another oxidizable group such that the reaction proceeds beyond the indophenol stage in the presence of persulfate. However, reduction with zinc dust followed by autoxidation caused the leuco base to go back to the indophenol stage only. A second addition of persulfate caused the reformation of the orange-red color observed in the first stage.

The nature of the reaction of ammoniacal persulfate on para substituted phenols was not investigated. It is unlikely that the para substituted products were oxidized to *o*-indophenols to any extent. Although the literature on these derivatives is scarce and many proposed structures are not based on solid evidence, Hodgson and Nicholson (*3*) definitely prepared 3,3′ difluoro-*o*-indophenol by the action of nitrous acid on *m*-fluorophenol. This compound is red in alkaline solution and can be reduced to the leuco form with zinc dust and acetic acid, which in turn is autoxidizable. Similar colors and behavior were not observed with the oxidization products obtained from the para substituted phenols.

Table I. Results of Color Tests

	A. Phenols with Free Para Positions[a]	Color before and after Reoxidation of Leuco Dye
1.	Acetylsalicylic acid	Light green
2.	*m*-Bromophenol	Deep green
3.	*o*-Bromophenol	Deep blue
4.	Butyl salicylate	Deep green
5.	6-Chloro-2-methylphenol	Deep blue
6.	*m*-Chlorophenol	Deep green
7.	*o*-Chlorophenol	Deep blue
8.	*m*-Cresol	Deep green
9.	*o*-Cresol	Deep blue
10.	2,4-Dihydroxybenzoic acid[b]	Deep blue-green
11.	*m*-Ethylphenol	Deep green
12.	*o*-Hydroxyacetanilide[c]	Deep green
13.	*o*-Hydroxyacetophenone	Light green
14.	2-Hydroxy-4-aminobenzoic acid	Orange
15.	*m*-Hydroxybenzaldehyde	Deep green
16.	*m*-Hydroxybenzoic acid[d]	Deep green
17.	*o*-Hydroxybenzyl alcohol	Deep blue-green
18.	*o*-Hydroxybiphenyl	Deep green
19.	2-Hydroxy-3-methoxybenzaldehyde	Deep green
20.	1-Hydroxy-2-naphthoic acid	Light green
21.	*o*-Iodophenol	Deep green
22.	*Mono*-6-*tert*-butyl-*m*-cresol	Deep green
23.	*o*-Nonylphenol	Deep green
24.	*m*-Pentadecylphenol	Deep green
25.	Phenol	Deep blue-green
26.	Phenyl salicylate	Deep green
27.	8-Quinolinol	Deep orange
28.	Resorcinol monoacetate	Green precipitate
29.	Salicylamide	Light green
30.	Salicylic acid	Deep green
31.	Salicylonitrile	Light green
32.	Thymol	Deep green
33.	2,6-Xylenol	Deep blue
	B. Phenols with Substituted Para Positions	
34.	Benzyl *p*-hydroxybenzoate	Orange
35.	*p*-Benzylphenol	Orange-yellow
36.	*p*-Bromophenol	Light green
37.	2-Chloro-5-hydroxytoluene	Deep green
38.	*p*-Chlorophenol	Light green
39.	4-Chloro-2-phenylphenol	Deep green
40.	2-Chloro-4-phenylphenol	Yellow
41.	5-Chlorosalicylic acid	Orange-yellow
42.	6-Chlorothymol	Orange-yellow
43.	*p*-Cresol	Light yellow

	B. Phenols with Substituted Para Positions	Color before and after Reoxidation of Leuco Dye
44.	*p*-(α-Cumyl) phenol	Yellow-brown precipitate
45.	*trans*-α,α′-Diethylstilbestrol	Deep yellow
46.	4,4′-Dihydroxydiphenyl	Brown-orange
47.	4,4′-Dihydroxydiphenyl sulfone	Deep orange
48.	2,6-Dimethyl-4-bromophenol	Yellow
49.	3,5-Dimethyl-4-chlorophenol	Deep yellow
50.	Ethyl *p*-hydroxybenzoate	Orange
51.	Meso-hexestrol	Deep yellow
52.	*p*-Hydroxyacetanilide[b]	Orange-yellow
53.	*p*-Hydroxyacetophenone	Orange-yellow
54.	*p*-Hydroxybenzoic acid	Yellow-orange
55.	*p*-Hydroxybenzophenone	Yellow
56.	*p*-Hydroxydiphenyl	Orange-yellow
57.	3-Hydroxy-2-naphthoic acid	Light yellow
58.	*p*-Hydroxypropiophenone	Light yellow
59.	Methyl *p*-hydroxybenzoate	Yellow
60.	5-Phenylsalicylic acid	Orange
61.	*n*-Propyl *p*-hydroxybenzoate	Orange-yellow
62.	2-Quinolinol	Light yellow
63.	Sulfosalicylic acid	Orange
64.	2,4,6-Tribromophenol	Deep yellow
65.	2,4,6-Trichlorophenol	Deep yellow

[a] Derivatives possessing hydroxy or amino groups ortho or para to a given hydroxy group reduced the silver ammonium ion to metallic silver. The compounds themselves were rapidly oxidized to quinhydrones, quinones, quinone imines, etc., which in turn undergo complex condensations in alkaline solution. Hence, in most of these cases, gray or brown solutions containing similarly colored precipitates resulted by the action of alkaline persulfate. Although *o*-nitrophenol gave a dark green color in the test, other nitro derivatives such as 2-nitroresorcinol, 3,5-dinitrosalicylic acid, and *m*-nitrophenol caused the reduction of silver ammonium ion and brown colorations.

[b] After the oxidation of this compound with persulfate the color is an orange-red instead of the usual blue or green. However, after the solution is reduced with zinc dust and reoxidized by air, it becomes blue-green.

[c] *o*- and *p*-Hydroxyacetanilides gave the typical colorations of their respective groups if they were not preheated with zinc in boiling alkali. Apparently, the oxidation proceeds rapidly in comparison with the hydrolysis of the acetyl group so that the latter remains intact. If these derivatives are first refluxed with zinc and alkali, the resulting solutions, consisting of the free *o*- and *p*-aminophenols, reduce silver ammonium ion.

[d] *m*-Hydroxybenzoic acid showed behavior opposite to 2,4-dihydroxybenzoic acid. On oxidation with persulfate the solution was green, but after reduction with zinc dust followed by autoxidation the solution became orange. The first stage color obtained in the oxidation of this compound is greatly improved when the reaction is carried out around 0° C.

The anomalous behavior of the para halosubstituted phenols requires further study. To make certain that the results observed were not due to impurities, many of these derivatives were recrystallized as many as six times from different solvents. The colors obtained from the most purified fractions showed no qualitative differences from the original samples. It may be concluded from the ten para halogenated phenols tried that if both the positions, ortho to either the halogen or phenol groups, are substituted, the compounds behaved like other phenols with blocked para positions. Other instances point up the importance of the nature of the group—e.g., 2-chloro-5-hydroxytoluene gave a positive test and 5-chlorosalicylic acid a negative one.

Zincke and coworkers (*5*) observed the migration of groups in the oxidative nitration of certain para substituted phenols. For example, 4-methyl-6-chlorophenol was oxidized with nitric acid to 2-nitro-4-chlorotoluquinone and/or 2-chloro-4-nitrotoluquinone. This work strengthens the hypothesis that the para halogen atom in certain phenols may migrate under oxidative conditions. Neither chloride ion nor oxidized ions of chlorine could be detected after the persulfate oxidation.

Escaïch's observation that hypobromite or hypochlorite ions can be substituted for persulfate ion was confirmed. *N*-Bromosuccinimide, *N*-chlorosuccinimide, and 1,3-dichloro-5,5-dimethylhydantoin are also usable and may offer some advantage if the test is to be carried out in a nonaqueous solution. The solubilities of inorganic persulfates are extremely low in these media. Sodium peroxide, potassium chlorate, potassium iodate, and potassium bromate are ineffective substitutes for potassium persulfate.

Many of the common metallic ions capable of existing in more than one valence state were tried in lieu of silver ion as catalysts. Cupric ion seemed to be the only effective substitute. Thorium(IV), cerium(IV), praseodymium(III), and neodymium(III) were of doubtful value.

The sensitivity of the test varied considerably with the compound. Although phenol could readily be detected in a 0.03% aqueous solution, the concentration of salicyclic acid had to be increased tenfold before an adequate color resulted. By studying details of the reaction environment such as temperature, and concentrations of the reactants, the sensitivity may be greatly increased.

Finally, the test was found to be of value in detecting persulfate ion in the presence of other oxidants incapable of giving the Escaïch reaction.

LITERATURE CITED

(1) Escaïch, A., *J. pharm. chim.*, **22**, 140 (1920).
(2) Gibbs, H. D., *Chem. Revs.*, **3**, 291 (1927).
(3) Hodgson, H. H., and Nicholson, D. E., *J. Chem. Soc.*, **1939**, pp. 1405–8.
(4) Singer, A. J., and Stern, E. R., ANAL. CHEM., **23**, 1511 (1951).
(5) Zincke, T., and coworkers, *Ann.*, **328**, 314 (1903); **341**, 313 (1905).

RECEIVED for review November 3, 1954. Accepted January 10, 1955.

Colorimetric Determination of Sulfate Ion

JACK L. LAMBERT, STANLEY K. YASUDA, and MORRIS P. GROTHEER
Department of Chemistry, Kansas State College, Manhattan, Kan.

A colorimetric procedure for determining sulfate ion in the range of 0 to 400 p.p.m. is described which uses an insoluble thorium borate–Amaranth dye reagent. Sulfate ion releases dye molecules from the solid reagent in direct proportion to its concentration and is determined indirectly as the concentration of the dye at 521 mμ. Potentially serious interferences by fluoride, phosphate, and bicarbonate ions are eliminated through the use of added lanthanum ion and a weak acid cation exchange resin. Seven water samples were analyzed by this method with the results in good agreement with standard gravimetric analysis.

THE determination of fluoride ion by the cellulose-supported thorium-Amaranth lake (*1*) suggested that a similar method could be used for the determination of sulfate ion. The reagent used is thorium borate treated with Amaranth dye, which releases dye molecules in proportion to the sulfate ion concentration in solution. Bicarbonate, phosphate, and fluoride ions interfere by reacting with the thorium-dye reagent to release dye into solution. Phosphate ion interferes at concentrations not ordinarily found in water. The addition of lanthanum ion removes the fluoride ion with little effect on the sulfate ion. Bicarbonate ion interferes, but is eliminated by passing the sample solution through Amberlite IRC-50(H) weak acid ion exchange resin. With the use of lanthanum ion and a cation exchange resin, sulfate ion exchanges stoichiometrically for the dye molecules with the thorium-dye reagent with no interference from other ions.

REAGENTS AND EQUIPMENT

Thorium borate–Amaranth reagent, ground to pass 200 mesh.

Standard sulfate solution, 400 p.p.m., 0.724 gram of potassium sulfate per liter of solution.

Lanthanum ion, 1000 p.p.m., 0.779 gram of lanthanum nitrate hexahydrate per 250 ml. of solution.

Weak acid ion exchange resin, Amberlite IRC-50(H), analytical grade.

Filter paper, Whatman No. 42 and 50.

Funnels.

Test tubes, 25 × 200 mm.

Interval timer.

Spectrophotometer, Beckman Model DU, 10-mm. cells.

Measuring spoon made from nickel double-end spatula having a diameter of 0.5 cm. (see Figure 1).

PREPARATION OF REAGENT

Thorium borate is obtained by the reaction of a 1-liter solution of 0.01*M* thorium nitrate and 0.05*M* sodium tetraborate, the latter being added dropwise with constant stirring. The thorium borate precipitates as a somewhat gelatinous white solid and settles to the bottom in several minutes. The solution is decanted and the precipitate is centrifuged to remove additional water. With the precipitate equally divided into two 250-ml. centrifuge cups, each portion is washed and centrifuged four times with 100 ml. of water. To each cup, 100 ml. of 0.2% Amaranth solution are added and shaken for 1 minute to allow thorough mixing. The excess dye is centrifuged off, and the solid washed five times with 100-ml. portions of water. After the third washing, the solution is colorless. Water adhering to the precipitate is removed by washing three times with 100 ml. of acetone. Drying is accomplished by heating the precipitate in the oven at 60° C. for 30 minutes, after which the precipitate is allowed to dry at room temperature for 2 hours. The dried product, which is deep red in color, is ground and sieved, and that which passes through a 200-mesh sieve is collected. The finely divided solid reagent is thoroughly mixed.

PROCEDURE

The sample is run through the column of ion exchange resin, Amberlite IRC-50(H), 26 cm. high in a 50-ml. buret. The resin is held firmly in place by borosilicate glass wool plugs, one being

placed just above the stopcock outlet and the other at the top of the column. The column is rinsed twice with distilled water and the water sample being analyzed is allowed to run through freely. The column is again filled with the water sample to the zero mark and the first 20.0 ml. is collected in a test tube. To this portion 1.0 ml. of 1000 p.p.m. lanthanum ion and two spoonfuls of thorium borate–Amaranth reagent are added. The solution is shaken vigorously for 1 minute and then filtered twice through Whatman No. 42 filter paper to remove all traces of suspended excess reagent. The color of the solution is directly proportional to the sulfate ion concentration. The absorbance measurements are obtained spectrophotometrically using 10-mm. cells in a Beckman Model DU spectrophotometer at 521 mμ.

DISCUSSION

The calibration curve for the sulfate ion was determined over the range of 0 to 400 p.p.m., as shown in Figure 2. The curve differed slightly for each batch of reagent, but was linear in each case. At each concentration, five determinations were made. The mean, range, and average deviation are indicated.

As the measuring spoon for the reagent was not standard equipment, a study was made to determine whether the amount of reagent added had any effects on the color intensity. It was found that the color was independent of the amount of reagent added and proportional only to the sulfate ion concentration in solution. In all cases, the reagent was added in excess. A study of the reaction at times between 1 and 10 minutes showed that time was not a factor.

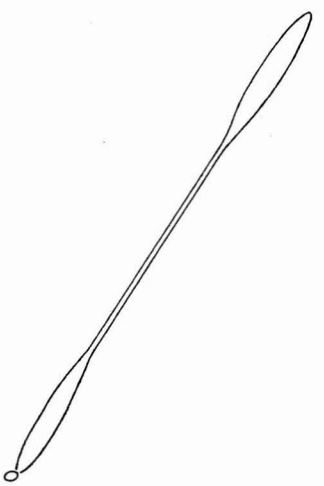


Figure 1. Measuring spoon

Possible interferences by ions normally found in water were investigated with the results shown in Table I. Bicarbonate, phosphate, and fluoride ions were the only ions which reacted with the thorium borate–Amaranth reagent. The addition of 1.0 ml. of 1000 p.p.m. lanthanum ion eliminated interference from fluoride up to 15 p.p.m. The bicarbonate ion which was found to be present in the waters analyzed was removed by the weak acid cation exchange column. Phosphate ion did not interfere at concentrations normally found in natural water. Other than these ions, no potential interferences were encountered. The concentrations listed with the ions show the maximum concentrations of those ions which could be present without interfering with the sulfate determination. Concentrations above 500 p.p.m. were not investigated.

In Table II, seven representative water samples were analyzed with the results in good agreement with those obtained from standard gravimetric analysis. For each sample, the suspended matters were removed by centrifuging and filtering through Whatman No. 50 filter paper with suction, and analyzed as described above.

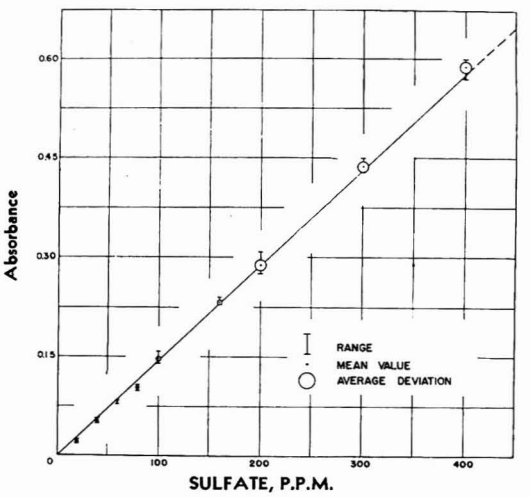


Figure 2. Calibration curve

Table I. Interference Study

(Sulfate ion absent)

Ion	Salt	Concentration, P.P.M.	Observation
Cl^-	NaCl	500	Colorless
HPO_4^{--}	$Na_2HPO_4.7H_2O$	25	Colorless
		50	Interferes
I^-	KI	500	Colorless
F^-	NaF	15	Colorless
NO_3^-	$NaNO_3$	500	Colorless
HCO_3^-	$NaHCO_3$	10	Colorless
		20	Interferes
Mg^{++}	$MgCl_2.6H_2O$	500	Colorless
Al^{+++}	$Al(NO_3)_3.9H_2O$	500	Colorless
Zn^{++}	$Zn(C_2H_3O_2)_2.2H_2O$	500	Colorless
Na^+	NaCl	500	Colorless
K^+	KCl	500	Colorless
Ca^{++}	$CaCl_2$	500	Colorless
NH_4^+	NH_4Cl	500	Colorless
Fe^{+++}	$FeCl_3$	500	Colorless
OH^-	NaOH	$10^{-4}N$	Colorless
		$10^{-3}N$	Interferes
H^+	HCl	$10^{-3}N$	Colorless

Table II. Water Analysis

Water Sample	Gravimetric Analysis[a], P.P.M. SO_4^{--}	This Method[a], P.P.M. SO_4^{--}
Manhattan, Kan.[b]	67	66
Smoky Hill River[c]	201	205
Junction City, Kan.[b]	66	71
Republican River[c]	145	145
Private well[d]	13	16
Wildcat Creek[e]	23	24
Kansas River[e]	82	83
Blue River[d]	49	55

[a] Average of two analyses.
[b] City water supply.
[c] Near Junction City, Kan.
[d] Near Manhattan, Kan.
[e] Near Ogden, Kan.

Using the proportions described for the preparation of the reagent, the yield was sufficient for 50 to 60 determinations. A number of batches may be made at the same time and thoroughly mixed after grinding and sieving. The standard curve for each thoroughly mixed quantity of reagent is determined by making several analyses on one or more standard sulfate ion solutions, and drawing the curve through the origin. The slight variations between batches may be due to the conditions of precipitation of the thorium borate and its reaction with the dye solution

ACKNOWLEDGMENT

This research was made possible through the aid of a grant from the Research Corp.

LITERATURE CITED

(1) Lambert, J. L., ANAL. CHEM., **26**, 558 (1954).

RECEIVED for review June 7, 1954. Accepted December 17, 1954.

Determination of Traces of Sulfur in Organic Compounds

Vertical Furnace Method

J. A. HUDY and R. D. MAIR

Hercules Experiment Station, Hercules Powder Co., Wilmington, Del.

A method is described for determining traces of sulfur in organic liquids. It is particularly suitable for the concentration range from 1 to 100 p.p.m. A vertical catalyst-packed furnace is used which is capable of burning from 15 to 30 grams of sample per hour. The sulfur is absorbed from the combustion gases in a hydrogen peroxide solution, after which the excess hydrogen peroxide is decomposed with the aid of a platinum catalyst. The resulting sulfate is then measured by a sensitive conductometric procedure. The method has been applied extensively to terpene hydrocarbons and appears applicable to many other types of compounds. It has a precision and accuracy of about 1 to 2 p.p.m. of sulfur.

A RAPID versatile method for the determination of small amounts of sulfur is essential in many investigations. For example, it is needed in problems that involve corrosion, catalyst poisoning, and the elimination of odors caused by sulfur compounds, or where the combined sulfur concentration may be less than 100 p.p.m. In many instances, the critical sulfur content is in the range of 1 to 10 p.p.m. In such cases, it may be important to determine sulfur in this range with a fair degree of accuracy.

Such problems have come up in this laboratory for many years, but existing methods of analysis have been unable to solve them. A search was begun several years ago for a method that would be versatile, sensitive, and practical. The method described in this paper is the result of that search.

The first requirement is a rapid and reliable means of converting the sulfur into a measurable form. This is usually done by burning the sample and determining the sulfur as sulfate. However, most existing combustion procedures have limitations which make them unsuitable for trace determinations. Conventional methods based on the oxygen and peroxide bombs (*1*) or combustion from a boat in a horizontal tube furnace (*3*) lack the necessary sensitivity because sufficient sample cannot be burned. The sensitivity of the ASTM lamp method (*2*) has been extended downward to 1 p.p.m. by purifying the air supply (*10*), but its utility has been restricted by a number of factors, including the limited variety of materials which can be burned without dilution and the extended time required for burning the sample.

A recent trend which leads to increased sensitivity has been the development of furnaces in which relatively large amounts of sample can be burned in a reasonable time. Strafford and Crossley (*7*), and more recently Holeton and Linch (*5*), have described horizontal spray furnaces of this type. Hagern an pioneered a vertical drip-type furnace (*4*) which is much more compact and simpler to construct and to operate than horizontal units. Hagerman's arrangement was improved upon by Wilson and Straw (*9*), by packing the combustion tube with an oxidation catalyst of supported vanadium pentoxide. These last authors made only limited attempts to determine sulfur in the lower concentration ranges. However, their apparatus appeared to offer a promising solution to the combustion problem in trace sulfur analysis, as it enabled samples with a wide range of volatilities and states of oxidation to be conveniently and rapidly burned.

A second requirement is a sensitive, specific, and precise method for measuring the sulfate produced by combustion of the sample. Titration of the sulfur as sulfuric acid has been widely used for speed and simplicity, but is not specific, because other acids may be present. Gravimetric determination as barium sulfate is suitable only in the higher ranges of sulfur content. Turbidimetric and nephelometric methods (*10*), although highly sensitive, usually lack precision and are not convenient because test conditions must be rigidly controlled. Microtitrations with a barium chloride solution and tetrahydroxyquinone as indicator (*8*) have been confined mainly to relatively large amounts of sulfate.

However, the differential conductometric method of Polsky (*6*), originally developed for water analysis, has much to recommend it. It is not only sensitive, specific, and precise, but is also rapid and convenient. In this procedure, the specific conductance of a known volume of solution is measured before and after the addition of a standard amount of barium chloride reagent. The sulfate concentration is then determined as a function of the observed change in conductivity. This procedure has a sensitivity of 100 γ of sulfate and is well adapted to the analysis of samples that do not form too great an excess of ions other than sulfate.

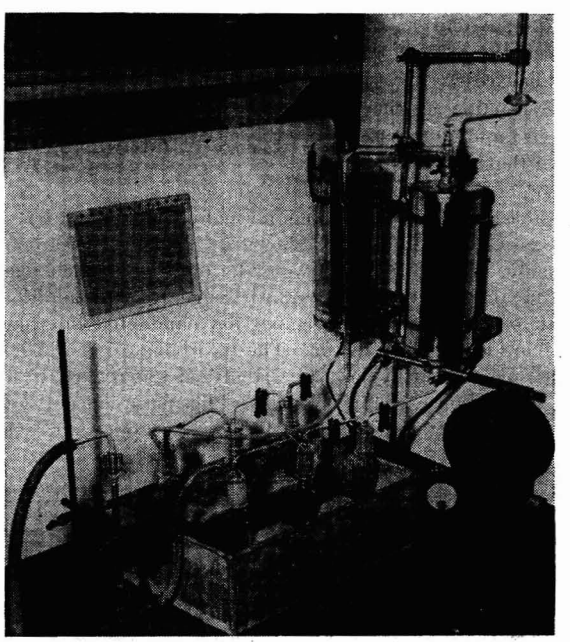


Figure 1. Combustion apparatus for determination of traces of sulfur

In the method described below, an improved version of the combustion apparatus of Wilson and Straw has been combined with the sensitive conductometric sulfate method of Polsky.

APPARATUS

The combustion apparatus, pictured in Figure 1, consists of a combustion furnace and an absorption train. Dual units are required. They are operated in series, the first unit serving to purify the air for the second unit in which the sample is burned.

Combustion Furnace. The outer shell of 16-gage aluminum is 12.5 inches long and 5 inches in diameter. The removable ends have 1.25-inch holes bored at the centers to admit the combustion tube. The Nichrome heating elements (commercially available from Arthur H. Thomas Co., Philadelphia, Pa., Catalog No. 5805) consist of two semicylindrical units connected in series. These are centered in the furnace shell by means of 0.25-inch Transite disks. The furnace is insulated with vermiculite. It is supported vertically on a heavy-based ring stand by means of two Vari-grip stainless steel beaker clamps. The combustion tubes consist of 14-inch lengths of 1-inch bore glazed quartz tubing with a 1.5-inch length of the inner member of a 12/5 spherical quartz joint sealed onto the constricted lower end.

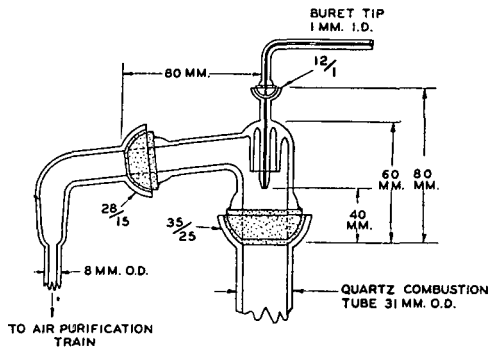


Figure 2. Diagram of enclosed sample feed system

The tube in which the sample is to be burned has the outer member of a 35/25 spherical quartz joint sealed to its upper end. The tube is supported at the lower end by an insulated ring. This allows it to be moved freely up or down in the furnace to control the temperature of the upper vaporizing zone. A combustion head for admitting the purified air and the sample is constructed as shown in Figure 2. The sample is introduced from a standard 50-ml. buret equipped with an 8-inch offset capillary tip. This tip is connected to the combustion head with a 12/1 spherical joint.

The temperature of the combustion tubes is regulated by a 20-ampere Variac which is connected in parallel with the dual furnaces. Temperatures from 800° to 850° C. at 100 to 115 volts are obtained with this arrangement and have proved satisfactory for most liquid samples boiling from 80° to 200° C. A thermocouple may be mounted in the furnace core to indicate the temperature, but it has been found more satisfactory to set the Variac at a predetermined voltage and to make periodic checks of the operating temperature with a pyrometer and a Chromel-Alumel thermocouple inserted into the combustion tube core.

Absorption Trains. The dual absorption trains each consist of three tall-form 125-ml. gas-washing bottles fitted with coarse-fritted gas dispersion tubes. Each absorber for the air purifying train contains 25 ml. of 10% hydrogen peroxide, and each absorber for the sample combustion train contains 25 ml. of 3% hydrogen peroxide. Connections between the furnace and absorbers are made with glass tubing sealed to 12/5 spherical joints. The air purifying train is connected to the sample combustion head with Tygon tubing. Both sets of absorbers are immersed in an ice bath. Air is drawn at 8 liters per minute through the furnaces by means of an aspirator. The rate is regulated by means of a rotameter (Emil Greiner Co., Catalog No. G-9145).

Conductance-Measuring Equipment. A Leeds and Northrup portable conductivity indicator (Catalog No. 4866), range 0.1 to 12,000 micromhos per cc., is used, with a dipping-type conductivity cell (Leeds and Northrup Catalog No. 4920), designed for low-conductivity solutions; cell constant about 0.1 per cm. A magnetic stirrer is also convenient.

CATALYST PREPARATION

The following method of catalyst preparation is essentially that of Wilson and Straw (*9*).

Heat 500 ml. of distilled water to about 80° C. in a 2-liter beaker in a hood. Add 100 grams of vanadium pentoxide (reagent grade, Vanadium Corp. of America) slowly with stirring. Continue heating the solution to about 90° C. and add 200 grams of reagent grade oxalic acid slowly with stirring until the vanadium is reduced and gives a clear blue solution of vanadium oxalate.

Pour the hot vanadium oxalate solution over 900 grams of alumina granules (grade T-71, 6 to 8 mesh, Aluminum Co. of America) in a stainless steel beaker. Mix well, pour off the excess solution, and evaporate to dryness on a hot plate with continuous agitation. Finally, ignite the product in air for 12 hours at 450° C. The catalyst should be shaken before use on an 8- to 10-mesh screen to remove vanadium pentoxide particles not adhering to the alumina.

Pack the combustion tubes as follows: Place a 2-inch layer of ignited coarse ($^3/_8$ to 0.5 inch) quartz chips at the lower end of the tube to support the catalyst. Add 8 inches of the catalyst, followed by a 2-inch layer of 0.25-inch quartz chips.

PROCEDURE

Sample Combustion. With the Variac set at 110 volts, bring the furnaces to maximum heat (850° C.) over a 1-hour period. To each of the three absorbers in the sample train add 25 ml. of 3% hydrogen peroxide solution (dilution of Merck Superoxol). Insert the absorber trains in the ice bath and connect them to the furnaces and aspirator. Fill the buret with sample and mount it on the furnace stand so that the tip is connected to the combustion head. Adjust the aspirator to give an air flow of 8 liters per minute through the system. Adjust the sample flow to a steady rate which is as rapid as will allow complete combustion. Adjust the temperature of the upper quartz chip layer by raising or lowering the combustion tube in the furnace until the sample vaporizes and burns smoothly. Both the sample flow rate and the position of the vaporizing zone depend on the volatility of the material being burned. For samples boiling in the range of 100° to 200° C. a feed rate of 20 to 30 drops per minute is suitable and the top of the quartz layer should be about level with the top of the furnace.

When enough sample has been burned to give an estimated minimum of 50 γ and a maximum of 1000 γ of sulfur, turn off the sample feed, note the volume of sample burned, and calculate its weight. Lower the combustion tube into the furnace and allow air to flow through the apparatus for 5 to 10 minutes at the same rate. When all of the visible sample is burned, reduce the air flow to 1 liter per minute and maintain it at this rate for an additional 20 minutes to ensure the complete removal of residual sulfur.

Disconnect the absorber trains and combustion head and empty the contents of the sample combustion train into a 250-ml. beaker, rinsing thoroughly with distilled water. Add a 1-inch-square piece of platinized 52-mesh platinum gauze, cover with a watch glass, and boil the solution at low heat until its volume is reduced to approximately 25 ml.; no further evolution of oxygen should be observed.

Transfer the peroxide-free absorber solution to a 50-ml. volumetric flask and dilute to volume with distilled water.

Sulfate Determination. Determining the sulfate concentration in the absorber solution by this method requires only two conductance measurements, which can be carried out in about 10 minutes. First, the initial specific conductance of the solution is measured. Then a fixed amount of a standard barium chloride solution is added to precipitate the sulfate, and the specific conductance is again measured. The observed increase in conductivity is inversely proportional to the sulfate concentration. Because of an ionic mobility effect, the conductivity increase is also dependent on the initial specific conductance of the sample, decreasing as the initial electrolyte concentration increases. Therefore, the observed conductivity difference must be corrected by adding a value obtained from a correction curve related to initial specific conductance. As shown by Polsky (*6*), this correction is relatively independent of the species of ion producing the initial conductance. The sulfate content is then obtained from a calibration curve of corrected conductivity difference *vs.* sulfate concentration.

Correction Curve. Prepare a solution of sodium chloride in distilled water having a specific conductance of about 700 micromhos (0.4 gram of sodium chloride per liter). Dilute 8-, 16-, 32-, 64-, and 125-ml. portions of this solution to 250 ml. with distilled water to form a series of six solutions with increasing conductivity. Measure the initial and final specific conductance (before and after adding barium chloride solution) of 50-ml. portions of distilled water and each of the sodium chloride solutions as described in the section on conductometric sulfate procedure. Subtract the initial conductivity from the final conductivity in each case to get the conductivity differences. Subtract the conductivity difference for each sodium chloride solution from that of distilled water to get the conductivity corrections. (The correction for distilled water will be zero.) Plot the conductivity correction for each solution *vs.* its initial conductivity. This is the correction curve (Figure 3, curve *A*).

Sulfate Calibration Curve. Accurately dilute a standardized solution of sulfuric acid to obtain a series of standard solutions containing about 2, 5, 10, 20, 40, and 50 p.p.m. of sulfate. Measure the initial and final specific conductance of 50-ml. portions of each solution. Subtract the initial conductivity from the final conductivity in each case to get the conductivity differences. Add to these differences the conductivity correction (Figure 3, curve *A*) which corresponds to the observed initial conductivity. This gives the corrected conductivity differences. Plot these values *vs.* the sulfate concentrations to obtain the sulfate calibration curve (Figure 3, curve *B*).

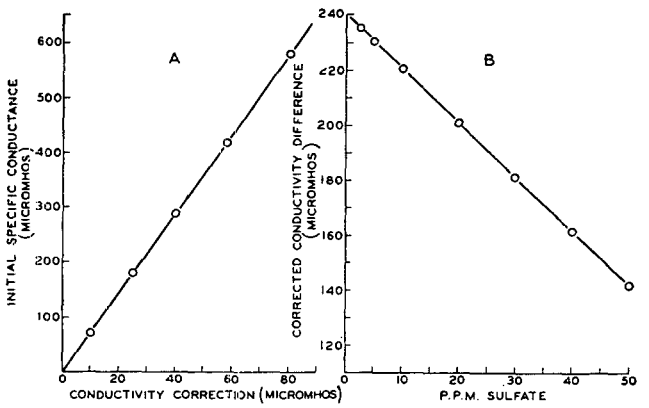


Figure 3. Sulfate calibration and correction curves

A. **Correction curve**
B. **Calibration curve (corrected)**

Conductometric Sulfate Procedure. To a 100-ml. beaker add the contents (50 ml.) of the flask containing the absorber solution from the sample furnace. Insert the conductivity cell into the solution, and measure the specific conductance of the solution with the conductivity bridge, dipping the cell up and down frequently and noting and correcting for temperature changes with the temperature compensator dial on the instrument. When the system reaches equilibrium (conductance readings become constant) record the specific conductance to the nearest micromho. (If this reading is over 600 micromhos, the solution may contain more than the estimated amount of sulfur and an appropriate dilution should be made.) Pipet 5 ml. of barium chloride reagent (2.54 grams of reagent grade barium chloride dihydrate per liter) into the solution and measure the specific conductance as before. Rinse the cell and beaker with distilled water between measurements.

Repeat the above procedure for a hydrogen peroxide reagent blank, which has been prepared in the same manner as the absorber solution.

Calculations. For each absorber solution, add the correction for ionic mobility, obtained from the correction curve, to the observed conductivity difference. Locate this corrected value on the sulfate calibration curve and note the corresponding sulfate concentration in parts per million. Calculate the micrograms of sulfur in the blank and in the sample, and the parts per million of sulfur in the sample as follows:

$$\text{Total micrograms of sulfur} = \frac{\text{p.p.m. } SO_4 \times 50}{3}$$

$$\text{P.p.m. of sulfur} = \frac{\gamma \text{ of sulfur in sample} - \gamma \text{ of sulfur in blank}}{\text{wt. of sample, grams}}$$

DISCUSSION

A preliminary study of the accuracy of the method was made by applying it to terpene fractions containing known added amount of sulfur, thiophene, and isobornyl sulfide. These results are presented in Table I.

The over-all recovery of the method at the levels studied is approximately 93%, which is satisfactory especially in the 1- to 20-p.p.m. range. The precision of the method is between 1 and 2 p.p.m. of sulfur at the 1- to 20-p.p.m. level.

Further evidence of the reproducibility of the method is shown by the results in Table II. These duplicate determinations over a wide range of concentrations show the reproducibility to be within 1 to 2 p.p.m., at the 1- to 10-p.p.m. sulfur level. At high levels, reproducibility is almost as good, frequently within 2 p.p.m. of sulfur.

Table I. Determination of Sulfur by Vertical Furnace Method

Sample	Sulfur, P.P.M. Added	Found
Terpene hydrocarbons	33.0	30, 33, 33
	28.1	29
Dipentene	25.0	26, 27, 22, 25
Terpene hydrocarbons	13.0	11, 12, 11, 9, 11
	13.2	12, 11, 11
Ethyl alcohol	12.8	12
Solvenol[a]	12.0	8, 9
Terpene hydrocarbons	8.3	9, 6, 9, 6
	7.0	7
	5.6	5, 3, 3
Solvenol	4.8	4
Terpene hydrocarbons	3.5	4, 1, 4, 4
	2.8	3, 2, 4, 3
	1.4	3, 1
	0	0, 0, 1
Solvenol	0	0, 1, 0
Dipentene	0	0, 0

[a] Monocyclic terpene hydrocarbons, Reg. U. S. Patent Office, by Hercules Powder Co.

In general, 20 to 30 ml. of sample may be burned per hour, although most alcohols and oxygenated materials may be burned more rapidly. Optimum feed rates vary with the type of sample, but in general, 1 drop every 3 seconds is satisfactory for samples boiling in the range of 100° to 200° C.

Only limited application of the method to resins and solids has been made. It was found that higher temperatures were necessary to burn off the carbonaceous residues remaining from some of these materials. Some satisfactory combustions have been run on solid samples, however, by dissolving them in suitable high-boiling solvents.

Table II. Determination of Sulfur

(Reproducibility of vertical furnace method)

Sample	Sulfur, P.P.M.
Benzene	206, 209
Cymene	182, 171
Cumene	
Sample A	83, 83
Sample B	57, 53
Sample C	51, 48
Sample D	5, 4
Sulfate Solvenol	
Sample A	82, 80
Sample B	38, 38, 39
Sample C	54, 53
Sample D	59, 61
Sample E	42, 43
Pinene	
Sample A	6, 5
Sample B	8, 10
Terpene hydrocarbons	
Sample A	3, 3
Sample B	6, 6
Sample C	1, 2
Sample D	3, 4
Dipentene	
Sample A	0, 0
Sample B	4, 2
Sample C	5, 3
Turpentine	
Sample A	3, 3
Sample B	2, 1
Solvenol	2, 3
Pinane	0, 0

Materials that contain metals capable of forming heat-stable sulfates cause difficulty, as do materials which are extremely volatile. However, highly volatile samples have been burned satisfactorily after dilution with a higher-boiling solvent of known purity. Because of the large ratio of air to sample, no difficulties have been encountered with explosions.

In the case of very volatile materials which must be burned directly, it is generally more satisfactory to operate the combustion units in parallel, using an open combustion system. In this system the extra furnace serves to determine a simul-

taneous air and reagent blank. The sample in this case is simply added dropwise into the open combustion tube from a modified buret with an offset capillary tip. Of course, this method is practicable only in locations having atmospheres with little or no sulfur contamination. The open tube combustion system has been found feasible when air contamination does not exceed 50 to 75 γ of sulfur per hour of operating time.

With the type of samples to which the method has been applied, the oxidation catalyst has been found to be satisfactory for over 1000 hours of operation. Some leaching of the catalyst has been noted when burning benzene or other materials which have high combustion temperatures, but in most cases, volatilization of the catalyst is no problem if proper sample feed rates are maintained.

Various types of alumina were tried as the catalyst support. A porous form of activated alumina (Alcoa Grade F-10) proved satisfactory after calcination at 1400° C. for 12 hours, to convert it to the nonadsorptive α-alumina and to remove sodium oxide. It has turned out to be simpler, however, to use Alcoa-Grade T-71, which is already calcined and very pure. This contains less than 0.02% sodium oxide. Several forms of tabular alumina have proved unsatisfactory because they lack the porosity which is essential to good catalyst support.

Absorption studies indicated that 90 to 95% of the sulfur oxides are absorbed in the first absorber and 5 to 10% in the second absorber. Cooling of the absorber system was found necessary to prevent excessive evaporation of absorbent; cooling also gave more efficient absorption of combustion gases.

It is necessary to decompose the hydrogen peroxide in the absorber solutions prior to the conductivity measurements. Otherwise, the peroxide interferes by decomposing at the electrodes of the conductivity cell. Platinum gauze has proved to be a most effective catalyst for this step, as it acts rapidly and introduces no contamination. A thin coating of platinum black on the gauze increases the speed of decomposition nearly fourfold. This coating is applied by electrolyzing the gauze in 3% chloroplatinic acid in the usual manner and polarizing it cathodically in dilute sulfuric acid.

The method is intended for sulfur determinations in the range of 1 to 100 p.p.m. However, it has been applied satisfactorily to samples containing as much as 0.1% sulfur by diluting the sample with isopropyl alcohol or some other suitable solvent. It has been used in these laboratories during the past 2 years for the analysis of several hundred samples of terpenes, aromatic hydrocarbons, and alcohols, and is finding wide use as a general method of sulfur determination.

LITERATURE CITED

(1) Am. Soc. Testing Materials, Philadelphia, Pa., "Book of ASTM Standards," Part 5, Method D 129-52, 1952.
(2) *Ibid.*, Method D 90-50T, 1952.
(3) Grote, W., and Krekeler, H., *Angew. Chem.*, **46**, 106 (1938).
(4) Hagerman, D. B., ANAL. CHEM., **19**, 381 (1947).
(5) Holeton, R. E., and Linch, A. L., *Ibid.*, **22**, 819 (1950).
(6) Polsky, J. A., *Ibid.*, **19**, 657 (1947).
(7) Strafford, N., and Crossley, H., *Analyst*, **60**, 163 (1935).
(8) Walter, R. N., ANAL. CHEM., **22**, 1332 (1950).
(9) Wilson, H. N., and Straw, H. T., *J. Soc. Chem. Ind.*, **69**, 79 (1950).
(10) Zahn, V., IND. ENG. CHEM., ANAL. ED., **9**, 543 (1937).

RECEIVED for review August 25, 1954. Accepted December 4, 1954. Presented before the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 2, 1954.

Color Reaction for Determination of Some Aromatic Nitro Compounds

CURT C. PORTER

Merck Institute for Therapeutic Research, Rahway, N. J.

Although the color reaction of di- and trinitro aromatic compounds with alcohol or acetone and alkali is well known, production of color with mononitro compounds has been only infrequently observed. *O*- and *p*-nitroaniline, *p*-nitrotoluene, and some of their derivatives produce orange, red, or purple colors in dimethylformamide upon the addition of tetraethylammonium hydroxide. The colors are sufficiently stable to provide a basis for the quantitative determination of several compounds.

THE production of red and blue colors by *m*-dinitro and trinitro compounds with acetone or alcohol and alkali may be utilized for identification and quantitative analysis (*2, 4, 6, 8, 10*). Mononitro compounds generally do not yield colors other than yellow under similar conditions. However, Bost and Nicholson (*2*) reported that 3-nitro-4-aminotoluene and its benzoate gave orange and red-orange colors with acetone and sodium hydroxide, and Carr (*3*) obtained a red color with nitrobenzene in acetone upon the addition of tetraethylammonium hydroxide (Teah). Under similar conditions Auerbach (*1*) observed the formation of a purple-red color with 2,3,5,6-tetrachloronitrobenzene.

The quantitative determination of some nitro compounds can be based upon the formation of yellow colors in alkaline solution. For example, Huggins and Smith (*5*) determined *p*-nitrophenol in alkaline solution by colorimetric comparison at λ = 420 mμ. Porter (*9*) used the yellow color produced in alcoholic sodium hydroxide for the determination of 4,4′-dinitrocarbanilide in chicken blood plasma. This type of reaction offers a simple means for the assay of biological material for certain nitro-aromatic drugs and may be carried out, if not in aqueous medium, in acetone or alcohol. However, biological materials normally contain substances which absorb light in the blue region of the spectrum, and interfere in the colorimetric determination of yellow-colored compounds.

It has been observed that certain mononitro compounds, principally derivatives of *o*- and *p*-nitroaniline and of *p*-nitrotoluene, develop orange, red, or purple colors in dimethylformamide (Dmfa) upon the addition of tetraethylammonium hydroxide. A number of the compounds examined yielded colors of sufficient stability to permit the construction of standard curves. The color reaction described should provide a basis for the determination of these as well as other compounds with similar structures.

EXPERIMENTAL

The reaction was carried out by adding 0.1 ml. of a 10% aqueous solution of tetraethylammonium hydroxide (Eastman Kodak Co., Rochester, N. Y.) to a solution of 5 to 100 γ of nitro compound in 9.9 ml. of dimethylformamide (Matheson, Coleman and Bell Co., East Rutherford, N. J.). For the Janovsky reaction, 10% sodium hydroxide and acetone were used in place of the above reagents.

Several solvents other than dimethylformamide were tried, but found to be of no value—i.e., they did not permit the development of the red or purple colors reported below. These solvents were formamide, ethyl formate, alcohol, acetone, and ethyl

acetate. The latter seems to be especially contraindicated, since the presence of a trace of it rapidly destroyed the color developed in dimethylformamide by tetraethylammonium hydroxide. Water should also be excluded, since it decreased the absorbance when added in moderate quantities—i.e., about 5% of the total volume.

Colors similar to those produced by tetraethylammonium hydroxide also resulted from the use of tetramethylammonium hydroxide; however, cloudiness frequently occurred in the solutions when the latter reagent was employed.

The amount of tetraethylammonium hydroxide used is fairly critical. When 0.1 ml. of less than about 5% reagent was used, the colors tended to fade more rapidly, the rate of fading being roughly inversely proportional to the strength of the reagent. Since tetraethylammonium hydroxide was available only as a 10% solution, higher concentrations were not tried. However, if undue instability is encountered when examining a compound, it is well to use more than 0.1 ml. of this solution. For example, the stability of color produced by *p*-nitrotoluene was appreciably increased by using 0.15 ml. rather than 0.1 ml. of tetraethylammonium hydroxide.

Spectrophotometric measurements were made with the Coleman Universal spectrophotometer, Model 14, and in a square, 1.3-cm. cuvette.

A number of the compounds, including the phenyl urea derivatives, were supplied by R. C. O'Neill, Research Laboratories, Chemical Division, Merck & Co., Inc. None of the compounds was further purified before use.

Table I. Color Reactions of Various Aromatic Nitro Compounds

No.	Dinitrobenzene Derivatives	Teah-DMFA: Wave length of max. abs., mμ	Teah-DMFA: Color	Teah-DMFA: Absorbance[a] at λ of max. abs.	Janovsky Reaction: Wave length of max. abs., mμ	Janovsky Reaction: Color	Janovsky Reaction: Absorbance[a] at λ of max. abs.
1	2,4-Dinitrotoluene	660 (410)	Blue	0.80	580 (670)	Blue	0.33 (0.23)
2	3,5-Dinitrobenzoic acid	545 (370)	Purple	1.23 (0.52)	570 (380)	Purple	0.27 (0.16)
3	*m*-Dinitrobenzene	524	Red	1.48	570	Purple	1.58
4	2,4-Dinitrophenylhydrazine	525	Red	1.09	410 (570)	Green	0.6 (0.3)
5	2,4-Dinitroanisole	490	Orange	0.74	578	Purple	0.42
6	3,5-Dinitrosalicylic acid	455	Yellow	0.68	398	Lt. yellow	0.40
7	2,4-Dinitro-1-naphthol	446	Yellow	0.76	445	Yellow	0.73
8	2,4-Dinitrophenol	430	Yellow	0.76	422	Yellow	0.73
9	2,5-Dinitrobenzoic acid	430	Yellow	0.09	385	Lt. yellow	0.03
10	*p*-Dinitrobenzene	430	Yellow	0.77	554 (400)	Red → yellow	0.27 (0.18)
11	2,4-Dinitrochlorobenzene	428 (520)	Yellow	0.59 (0.14)	550 (660)	Purple	0.58 (0.28)
	Mononitro Compounds						
12	2-Nitrodiphenylamine	550	Purple	0.51	430	Yellow	0.32
13	*p*-Nitrotoluene	520	Purple[b]	1.32	...	None	...
14	2-Nitro-4-chloroaniline	510	Red	0.57	417	Yellow	0.32
15	2-Nitro-4-chlorophenylurea	510	Red	0.53	416	Yellow	0.23
16	*o*-Nitroaniline	504	Red	0.68	407	Lt. yellow	0.38
17	4-Nitro-*o*-phenylenediamine	503	Red	1.97	408	Lt. yellow	0.69
18	*o*-Nitrophenylurea	502	Red	0.57	383	Lt. yellow	0.17
19	*o*-Nitroxylidine	500	Red orange	0.69	417	Yellow	0.34
20	*p*-Nitrophenylbiguanide	495	Orange	1.94	395	Lt. yellow	0.58
21	4-Nitrocarbanilide	480	Deep yellow	1.76	360	Lt. yellow	0.19
22	4-Nitro-4'-chlorocarbanilide	480	Deep yellow	1.45	460	Yellow	0.80
23	5-Nitro-2-aminoanisole	478	Deep yellow	1.93	395	Lt. yellow	1.11
24	*m*-Nitrophenol	478	Yellow	0.17	400	Lt. yellow	0.08
25	1-Benzoyl-3-(*p*-nitrophenyl)urea	475	Yellow	0.98	462	Yellow	0.90
26	4-Nitro-4'-cyanocarbanilide	473	Yellow	1.54	450	Yellow	0.58
27	4-Nitro-2-chloroaniline	470	Yellow	1.53	465	Yellow	0.51
28	2,6-Dichloro-4-nitroaniline	470	Yellow	1.41	465	Yellow	1.42
29	*m*-Nitrophenylurea	470	Lt. yellow	0.06	370	None	0.03
30	*p*-Nitrophenylurea	466	Deep yellow	1.04	446	Yellow	0.70
31	*m*-Nitrobenzaldehyde	465	Yellow	0.35	...	None	...
32	*p*-Nitroaniline	465	Deep yellow	1.06	386	None	0.51
33	1-Nitronaphthalene	462	Yellow[c]	0.60	...	None	...
34	8-Nitroquinoline	460	Yellow[d]	0.21	...	None	...
35	*o*-Nitrobromobenzene	460	Yellow[e]	0.13	375	None	0.04
37	*o*-Nitrophenol	452	Yellow	0.52	442	Yellow	0.50
38	*o*-Nitrochlorobenzene	450	Yellow[e]	0.17	...	None	...
39	4-Nitrophenylurethane	450	Yellow	0.99	436	Yellow	0.90
40	6-Nitroquinoline	440	Yellow	0.79	415	Yellow[c]	0.45
41	*p*-Nitrophenol	430	Yellow	0.93	424	Yellow	0.90
42	*p*-Nitrochlorobenzene	430	Yellow	0.75	...	None	...
43	4-Nitro-2-amino toluene	420	Lt. yellow[e]	0.50	390	Lt. yellow	0.13
44	*o*-Nitro-toluene	410	Lt. yellow	0.08	...	None	...
45	6-Nitrobenzimidazole	404	Lt. yellow[e]	0.71	392	Lt. yellow	0.40
46	2-Nitroresorcinol	400	Yellow[e]	0.22	488	Lt. orange	0.17
47	*m*-Nitroaniline	400	Lt. yellow	0.11	385	Lt. yellow	0.11
48	*o*-Nitrobenzaldehyde	400	None	0.04	...	None	...
49	*p*-Nitrobenzamide	380	None	0.12	360	None	0.02
50	5-Nitrobenzotriazole	386	Lt. yellow	0.32	382	Lt. yellow	0.26
51	5-Nitrouracil	385	Lt. yellow	0.50	370	None	0.20
52	*p*-Nitrobenzaldehyde	360	None	0.07	...	None	...
53	*p*-Nitrobenzoic acid	360	None	0.04	...	None	...
54	*p*-Nitroanisole	360	None	0.05	...	None	...
55	*m*-Nitroanisole	360	None	0.04	...	None	...
56	*o*-Nitrobenzoic acid	...	None	...	...	None	...
57	*m*-Nitrotoluene	...	None	...	...	None	...
58	Nitrobenzene	...	None	...	...	None	...
	Compounds Containing Two Nitro Groups on Separate Aromatic Rings						
59	4,4'-Dinitrodiphenylmethane	722 (460)	Blue-green	1.04	750 (490)	Blue	0.69
60	4,4'-Dinitrostilbene	596 (375)	Blue	0.53 (0.52)	...	None	...
61	4,4'-Dinitrocarbanilide	556	Purple	1.9	460	Yellow	0.48
62	4-Nitrobenzoyl-4'-nitrophenylhydrazine	522 (660)	Purple	0.8 (0.67)	547	Purple	1.19
64	4',3-Dinitrobenzanilide	450	Yellow	0.87	440	Yellow	0.83
65	3',4-Dinitrobenzanilide	415	Yellow	0.3	400	Yellow	0.25
66	3,3'-Dinitrobenzanilide	375	Yellow	0.12	375	Lt. yellow	0.11
67	2,4'-Dinitrobiphenyl	...	None	...	...	None	...

[a] 10γ compound/ml. solution. Light path, 1.3 cm.
[b] Required 0.15 rather than 0.1 ml. Teah for stable color.
[c] After standing 1 hour.
[d] After standing 0.5 hour.
[e] Increases with time.

RESULTS

The data in Table I show that *m*-dinitro compounds yielded colors with both tetraethylammonium hydroxide in dimethylformamide and with the Janovsky reagents. The tints varied somewhat, and in two cases were quite different for the two reactions.

None of the mononitro compounds tested gave more than a yellow color with the Janovsky reagents, while *o*- and *p*-nitroaniline and some of their derivatives yielded deep yellow to red and purple with dimethylformamide and tetraethylammonium hydroxide. The *o*-nitroaniline derivatives absorbed light at longer wave lengths than the *p*-derivatives, but the latter produced more intense colors. With the exception of *p*-nitrotoluene, which produced a purple color with dimethylformamide and tetraethylammonium hydroxide, mononitro compounds other than the aniline derivatives yielded yellow or colorless solutions. Some of these had absorption peaks centered at wave lengths between 440 and 480 mμ, but low absorptivity—e.g., *m*-nitrophenol and *m*-nitrophenylurea. A few compounds, such as 1-nitronaphthalene, 8-nitroquinoline, and *o*-nitrobromobenzene, developed yellow color upon long standing with dimethylformamide and tetraethylammonium hydroxide.

The compounds which contained two nitro groups on separate aromatic rings yielded rather remarkable results. 4,4′-Dinitrodiphenylmethane gave a blue color with either dimethylformamide and tetraethylammonium hydroxide or acetone and sodium hydroxide. 4,4′-Dinitrostilbene and 4,4′-dinitrocarbanilide were blue and purple, respectively, in dimethylformamide and tetraethylammonium hydroxide, but colorless or yellow in acetone and sodium hydroxide. *p*-Nitrobenzoyl-*p*′-nitrophenylhydrazine was purple under either set of conditions. A number of compounds, including *o*-phenylenediamine and nonnitrated carbanilide derivatives, not listed in the table, failed to yield color under the conditions described.

In order for the reaction to be applied to the analysis of biological material, the color produced should have an absorption maximum above about $\lambda = 450$ mμ, and an absorbance at the maximum of about 0.05 per γ per ml. or more, in the 13-mm. cuvette. A few compounds which met these criteria are listed in Table II. These data show that for the selected compounds, the useful range of concentrations is from about 0.2 to 5 γ per ml. The table also indicates the times after the addition of Teah to the dimethylformamide solutions during which colorimetric readings were reliable. Varying rates of color development and degrees of stability are represented—for example, the color with 4-nitro-*o*-phenylenediamine developed rapidly, but decreased at such a rate that the time of reading must be carefully standardized. *o*-Nitroaniline also produced color rapidly, but faded more slowly, so that readings taken 1 to 3 minutes after addition of tetraethylammonium hydroxide did not differ by more than 1%. At the other extreme was 4,4′-dinitrostilbene, the color of which reached a maximum after some 20 minutes, then remained steady for another 20 minutes.

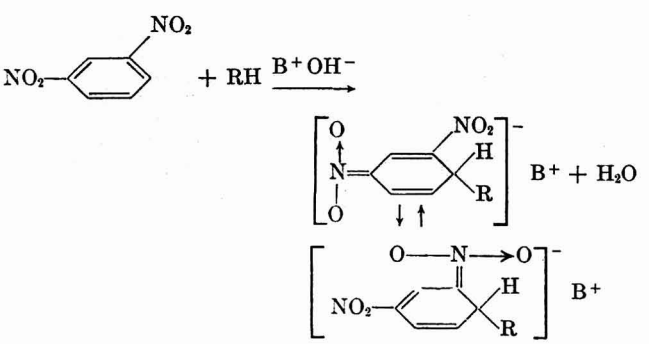
Table II. Some Compounds Amenable to Quantitative Determination

No.	Compound	Wave Length, Mμ	Time of Readings after Addition of Teah, Min.	Av. Absorbance[a] per γ per Ml., ± S.E.
62	4-Nitrobenzoyl-4′-nitrophenylhydrazine	660	1–2	0.066 ± 0.0014
60	4,4′-Dinitrostilbene	596	20–40	0.053 ± 0.0010
61	4,4′-Dinitrocarbanilide	556	1–2	0.189 ± 0.0036
12	2-Nitrodiphenylamine	550	1–10	0.051 ± 0.0001
14	2-Nitro-4-chloroaniline	510	1–3	0.057 ± 0.0013
15	2-Nitro-4-chlorophenylurea	510	4–20	0.053 ± 0.0012
16	*o*-Nitroaniline	504	1–3	0.068 ± 0.0013
17	4-Nitro-*o*-phenylenediamine	503	1	0.197 ± 0.0025
18	*o*-Nitrophenylurea	502	4–20	0.057 ± 0.0010
19	*o*-Nitroxylidine	500	1–10	0.069 ± 0.0019
20	4-Nitrophenylbiguanide	495	4–20	0.194 ± 0.0026
21	4-Nitrocarbanilide	480	1–10	0.176 ± 0.0031
22	4-Nitro-4′-chlorocarbanilide	480	1–5	0.145 ± 0.0020
23	5-Nitro-2-aminoanisole	478	1–20	0.193 ± 0.0025

[a] Light path, 1.3 cm.

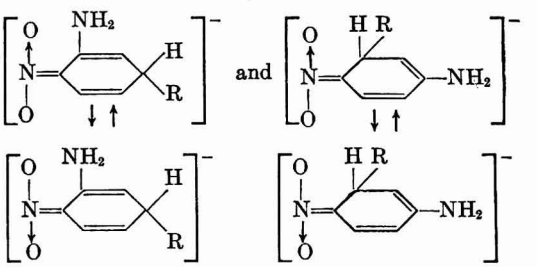
MECHANISM OF COLOR PRODUCTION

The reaction of *m*-dinitro and trinitro aromatic compounds with acetone or alcohol in the presence of strong alkali produces *o*- and *p*-nitro quinoid ions (*7*, *11*, *12*):



Taylor and Baker (*11*) suggested that the colors displayed by these products depend upon their existence as resonance hybrids.

It seems reasonable to assume that properly substituted mononitro compounds would react similarly with active hydrogen compounds, in the presence of alkali, to produce nitroquinoid ions such as the following:



Substituents on the molecule, other than the nitro group, may influence the reactivity of the compound, or alter the resonance of the nitro group in the quinoid ion. However, it cannot be stated with certainty at present whether either or both of these explanations account for the differences in color produced by various mononitro compounds with reactive hydrogen compounds and base.

LITERATURE CITED

(1) Auerbach, M. E., ANAL. CHEM., **22**, 1287 (1950).
(2) Bost, R. W., and Nicholson, F., *Ibid.*, **7**, 190 (1935).
(3) Carr, J. J., *Ibid.*, **25**, 1859 (1953).
(4) English, F. L., *Ibid.*, **20**, 745 (1948).
(5) Huggins, C., and Smith, D. R., *J. Biol. Chem.*, **170**, 391 (1947).
(6) Janovsky, J. V., *Ber.*, **24**, 971 (1891).
(7) Meisenheimer, J., *Ann.*, **323**, 205 (1902).
(8) Parks, G. D., and Farthing, A. C., *J. Chem. Soc.*, 1275 (1948).
(9) Porter, C. C., unpublished data.
(10) Rudolph, O., *Z. anal. Chem.*, **60**, 239 (1921).
(11) Taylor, T. W. J., and Baker, W., "Sidgwick's Organic Chemistry of Nitrogen," p. 260, Oxford University Press, London, 1937.
(12) Zimmerman, W., *Z. physiol. Chem.*, **245**, 49 (1936).

RECEIVED for review November 2, 1954. Accepted December 20, 1954.

Specific-Surface Determination of Nitroguanidine by Microscopic and Air-Permeability Methods

A. S. BASS and H. M. STERNBERG[1]

U. S. Naval Powder Factory, Indian Head, Md.

By the introduction of area and perimeter shape factors, the specific surface of acicular nitroguanidine crystals of irregular cross section was determined microscopically. This value was compared with those obtained with two routine air-permeability pieces of equipment. It was determined that the air-permeability equipment could validly be used for the routine specific-surface determination of needlelike nitroguanidine crystals in the range 7000 to 9000 sq. cm. per cc.

AN INVESTIGATION has been made to determine whether the specific surface of crystals of nitroguanidine can be measured in a routine manner, with either the Fisher subsieve-sizer or the draft equipment described in a joint Army-Navy specification (*2*). The equation, K, on page 9 of this specification contains errors and should read:

$$K = \frac{2\,pc}{2.303X(\log h_1 - \log h_2)\left[\frac{2v}{X} + \frac{pc}{c_1} + \frac{2(h_1 - h_2)}{2.303\,(\log h_1 - \log h_2)} - h_1\right]}$$

The operation of both pieces of equipment is based on the principle that the finer the particle size, the more difficult it is for air to permeate the sample bed. The precision with both pieces of equipment is very good, and excellent checks are obtained within certain permissible tolerances. The Fisher subsieve-sizer, for example, does not claim an accuracy within ±10%.

For a product containing needlelike crystals, such air-permeability equipment could be used to control the size of the crystals, although the values obtained might not be true values, provided the properties of the product remain within an acceptable tolerance range. However, the degree of accuracy of such instruments is uncertain, since the values obtained may be far from the true value, especially for acicular crystals of irregular cross section typified by nitroguanidine. Because nitroguanidine is highly aspherical, the shape factor becomes important. Drinker and Hatch (*1*) refer to the effect of shape factor upon the ultimate size of irregular particles and point out "that the frequent assumption of spherical shape for dust particles is not in accordance with facts."

It was decided to obtain a third measurement of specific surface with which to compare the values found with the air-permeability equipment. An equation for specific surface was derived based on the periphery or shape of the crystals, and a particle-size determination was made microscopically. Although every crystal irregularity cannot be considered with the microscope, it nevertheless can be used to make direct crystal measurements with either an ocular micrometer or by photographic enlargement and subsequent projection onto a screen where measurements can be made more conveniently and with less eye fatigue. This latter procedure was adopted and a nitroguanidine lot containing conventional, acicular crystals was used for this determination.

DERIVATION OF SPECIFIC-SURFACE EQUATION

The nitroguanidine crystals were treated as right cylinders of irregular cross sections.

Let:

- S = specific surface (ratio of square centimeters of surface to cubic centimeters of volume)
- s = crystal surface (surface area in square centimeters)
- v = crystal volume
- d = projected diameter of crystal cross section
- h = crystal length
- α = cross-section perimeter shape factor (ratio of perimeter to diameter)
- β = cross-section area shape factor (ratio of area to square of diameter)
- c = ratio of crystal length to diameter

Then

- αd = perimeter of crystal cross section
- βd^2 = area, A, of crystal cross section
- dc = length, h
- $\alpha cd^2 + 2\beta d^2$ = surface, s
- $c\beta d^3$ = volume, v

$$\text{Specific surface } (S) = \left(\frac{\alpha}{\beta} + \frac{2}{c}\right)\frac{\Sigma d_n^2}{\Sigma d_n^3} \qquad (1)$$

If the ratio of length to diameter, c, is large, it can be neglected without introducing any appreciable error, and Equation 1 reduces to

$$S = \frac{\alpha}{\beta} \times \frac{\Sigma d_n^2}{\Sigma d_n^3} \qquad (2)$$

PROCEDURE

An acetone slurry of clear cellulose acetate plastic plus 5% by weight of suspended nitroguanidine was extruded through a die under a pressure of 600 pounds per square inch, giving a strand approximately 0.15 inch in diameter. At this pressure the nitroguanidine crystals become oriented lengthwise in the direction of extrusion. Transverse sections (about 10 microns) of these strands were prepared with a microtome and then were photomicrographed at 950×. Figure 1 shows a typical photomicrograph. These photomicrographs were then projected onto a screen, and the outlines of the crystal cross sections traced.

Measurements were made on 200 crystals to find the projected diameter of cross section, the square root of the area of

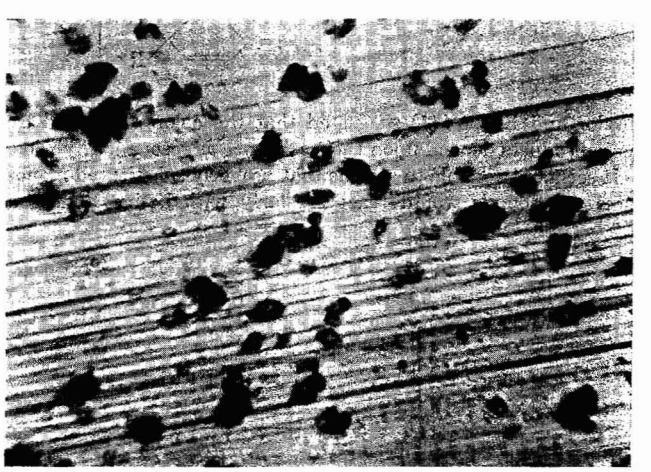


Figure 1. Photomicrograph showing cross sections of nitroguanidine crystals suspended in cellulose acetate plastic

515×

[1] Present address, U. S. Naval Ordnance Laboratory, White Oak, Silver Spring, Md.

cross section, the perimeter of cross section, and the length. The cross-section perimeters and areas were measured with a map measure and a planimeter, respectively. The remaining measurements were made microscopically, the length being measured on unimbedded nitroguanidine crystals, such as are shown in Figure 2, and the projected diameter being measured on the transverse sections of the strands.

Frequency-distribution curves of the data were made, and from these curves the shape factors, α and β, were obtained by comparing the perimeter and square root of the area, respectively, with the projected diameters. The ratio of crystal length to diameter was similarly obtained.

RESULTS

Frequency distributions of the data on the nitroguanidine crystals are shown in Figure 3. The values on the abscissa of Figure 3 represent (in per cent) the number of particles less in diameter, square root of area, length, or cross-section perimeter than the corresponding measurement in microns on the ordinates. These graphs give the following values for the constants:

$$\alpha = 3.41$$
$$\beta = 0.73$$
$$c = 20.6$$

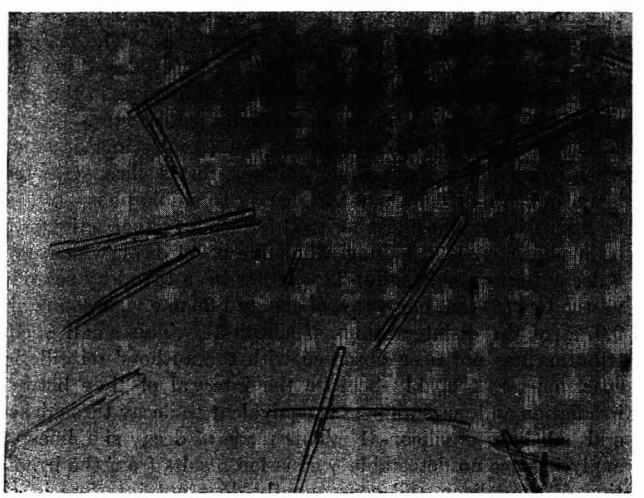


Figure 2. Photomicrograph of nitroguanidine crystals showing relative dimension of longest axis (length of crystal)

220×

The above values for the constants and the data from the cross-section-diameter distribution shown in Figure 3 were substituted in Equation 1 to obtain a value for the specific surface. The average specific surface as determined with the three instruments and the per cent deviation from the microscopic value are as follows:

Instrument	Specific Surface, Sq. Cm./Cc.	% Deviation from Microscope Value
Microscope	6702	...
Fisher subsieve sizer	8460	26.2
Draft equipment	8910	32.9
Average of Fisher subsieve sizer and draft equipment	8685	29.6

The values obtained with the Fisher subsieve sizer and the draft equipment are in reasonably close agreement, as would be expected, inasmuch as they both depend on the air-permeability principle, but the respective values for the two air-permeability instruments do not indicate such close agreement with the value obtained microscopically. However, the apparent difference in the values obtained with the two types of instruments (microscopic and air-permeability) is not as great as the results might indicate. The true specific-surface value is probably intermediate between the microscopic and air-permeability values. The microscopic value is probably on the low side of the true value, as fissures and minor surface irregularities cannot be taken into account in making direct measurements. Furthermore, an accuracy within ±10% is not claimed for the Fisher subsieve sizer and the value actually obtained with this instrument could be as much as 10% lower than that noted above. These considerations would minimize the difference in values obtained with the two types of instruments. All the measurements, therefore, may be considered as within a practical range of the true value, and the air-permeability equipment may be used for the routine determination of the specific surface of nitroguanidine within the range of 7000 to 9000 sq. cm. per cc.

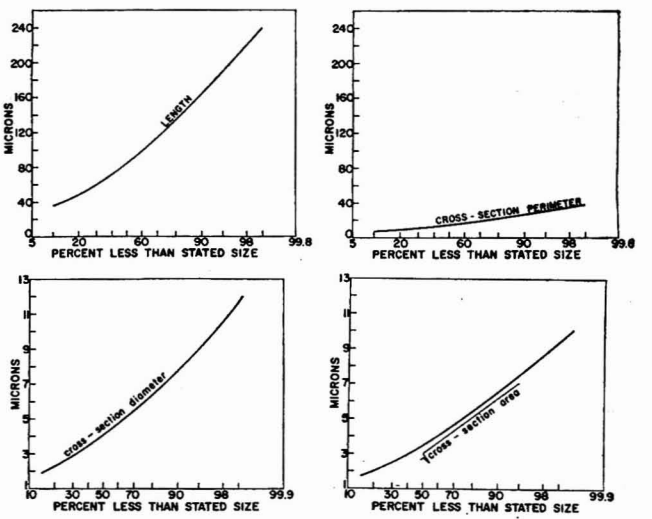


Figure 3. Frequency distribution of crystal lengths, cross-section perimeters, cross-section diameters, and square roots of areas

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of C. N. Bernstein, Leon Whitman, and Bryan Hancock, who furnished the specific-surface values as obtained with the air-permeability instruments.

LITERATURE CITED

(1) Drinker, P., and Hatch, T., "Industrial Dust," McGraw-Hill, New York, 1936.
(2) Joint Army-Navy Specification, "Nitroguanidine (Picrite)," JAN-N-494 (Sept. 10, 1947).

RECEIVED for review October 25, 1954. Accepted January 21, 1955.

Reliability of Routine Molecular Weights Determined by Light Scattering—Correction

In the article "Method for Evaluating the Reliability of Routine Molecular Weights Determined by Light Scattering" [Mastrangelo, S.V. R., Clay, Barbara, Fishman, M. M., Hagan, A. G., Lazrus, Allan, and Zagar, Walter, ANAL. CHEM., **27**, 262 (1955)] the expression in Equation 1 and the paragraph above Equation 1 should read: H^c/τ. Equations 9 and 10 should read $\beta/2$, not β^2. On page 264, second column, below Table II, the expression in line 3 should read: $M_{ge}{}^{0.75\beta^2}$.

Activation Analysis of Trace Impurities in Silicon Using Scintillation Spectrometry

GEORGE H. MORRISON and JAMES F. COSGROVE

Chemistry Laboratories, Sylvania Electric Products, Inc., Bayside, N. Y.

A method has been developed for the quantitative determination of trace impurities in silicon based on the use of neutron activation analysis. Gamma scintillation spectrometry and other physical methods for the measurement of radiations have been employed to identify and measure the amounts of the various impurities present. These physical methods minimize chemical manipulations, thereby eliminating loss or contamination during the course of the trace analysis. The accuracy and precision of the method were determined by analysis of an NBS standard aluminum alloy. A sensitivity of 0.001 to 1 γ is attained for most elements using this technique.

THE use of neutron activation analysis provides a valuable tool for the determination of subspectroscopic quantities of impurities in a variety of materials. The present study is based on the use of this technique in conjunction with scintillation spectrometry and other physical methods for the measurement of radiations. A minimum of chemical manipulations is involved in the course of this trace analysis, thereby eliminating the possibility of loss or contamination.

Since the electrical properties of semiconductors are markedly influenced by the presence of extremely small amounts of impurities, this method was applied to the analysis of trace impurities in silicon. It is interesting to note that as little as 1 impurity atom in 10^9 atoms of germanium affects transistor behavior. Previously available methods of trace analysis have proved ineffective in this connection.

When irradiated with thermal neutrons in a nuclear reactor, many impurities undergo an n, gamma reaction with the formation of the corresponding radioactive isotopes of these impurity elements. These radioactive species are then identified by means of the energies of the resultant radiations (β, γ) and the half lives of the radionuclides. The principles of activation analysis have been reviewed by Boyd (*1*), Leddicotte and Reynolds (*5*), and Taylor and Havens (*9*), and the analysis of radionuclide mixtures using scintillation spectrometry has been described by Connally and Leboeuf (*2*).

EXPERIMENTAL

Silicon Samples. The samples analyzed in this study consisted of high-purity commercially available polycrystalline silicon prepared by several different processes. Samples 1, 2, and 3 were obtained from different batches prepared by zinc reduction of silicon tetrachloride (*7*). Sample 4 had been prepared by the thermal decomposition of silicon tetraiodide on tantalum wire (*6*). A single crystal grown from silicon prepared by the zinc reduction process was also analyzed. This crystal, obtained from the Squier Signal Corps Laboratories, had been grown by the floating zone technique (*4*).

Comparative Standards. As a comparative method was employed to permit quantitative determination of various impurities, weighed amounts of pure metals or the appropriate compounds were employed for eventual comparison with the unknowns.

Aluminum Standard Sample. To test the accuracy and precision of the method for trace amounts of impurities, samples of aluminum with known impurity content were analyzed. A sample of certified National Bureau of Standards aluminum-base alloy 86-C served for a test of accuracy.

Irradiation. A sample of appropriate size (0.05 to 1 gram), depending upon the amount available and the anticipated purity of the material, together with the corresponding pure elements used as comparative standards, were sealed in separate quartz ampoules for irradiation in the Brookhaven pile. Upon irradiation for 3 days at a flux of approximately 3.4×10^{12} neutrons per sq. cm. per second, most of the elements of the periodic table produce radioactive species. The maximum flux of the Brookhaven reactor was employed to produce as high an activity as possible from the trace impurities for a 3-day irradiation. This time of irradiation was chosen for convenience. The majority of these elements produce one or more radionuclides which emit γ photons which can be identified by the differences in the energies of these photons using a scintillation spectrometer.

Silicon, the major component of the samples in this study, when irradiated under these conditions, forms silicon-31, which has a half life of 2.6 hours and decays to stable phosphorus-31 by β emission. The phosphorus-31 formed is further activated to form phosphorus-32, having a half life of 14 days, which upon β decay forms stable sulfur-32. It has been found by some investigators (*8*) that one γ ray, of energy 1.26 m.e.v., accompanies 0.07% of the disintegrations of silicon-31. This small γ contribution has not been observed with the authors' scintillation spectrometer, probably because the interval of time between irradiation and measurement is equivalent to decay through several half lives of silicon-31. This mode of decay simplifies the analysis since no detectable γ emission results from the irradiation of the silicon and consequently does not interfere in the γ scintillation scans. The nuclear data for the irradiation of silicon are listed in Table I.

Table I. Nuclear Data for Irradiation of Silicon

Element	Stable Isotope	Natural Abundance, %	Isotopic Cross Section, Barn	Radioactive Isotope Produced	Half Life	Method of Decay	Isotope Formed
Si	Si^{28}	92.21	0.08	..	....	..	....
	Si^{29}	4.70	0.3	...	....	...	....
	Si^{30}	3.09	0.2	Si^{31}	2.6 h	β'	P^{31} (stable)
P	P^{31}	100.0	0.19	P^{32}	14 d	β'	S^{32} (stable)

Preparation for Counting. After irradiation the silicon samples were given a surface leach with a solution prepared from equal volumes of saturated potassium hydroxide and 30% hydrogen peroxide to remove any possible surface contamination due to sampling and handling prior to irradiation. The samples were then crushed and mounted in aluminum planchets of 0.738 gram per sq. cm. thickness to absorb the maximum energy β particles given off by the silicon (1.47 m.e.v.). The simultaneously irradiated standards were completely dissolved in appropriate solvents after the addition of carrier and diluted to known volumes in volumetric flasks. Suitable aliquots of the standard radioactive solutions were taken for obtaining the relationship of activity to mass of the various elements determined. These

aliquots were evaporated to dryness and were ready for measurement of gamma activity using the spectrometer.

The standard aluminum samples were completely dissolved after irradiation and diluted to known volumes. As the authors were interested in the measurement of trace amounts of impurities, appropriate aliquots were taken, evaporated to dryness, and compared with pure standards using the scintillation spectrometer. Since the aluminum-28 formed from irradiation of the matrix has a half life of 2.3 minutes, radioactive measurement of the impurities of interest was performed after an elapsed period of time sufficient to permit decay of the aluminum, thereby eliminating any contribution by the matrix.

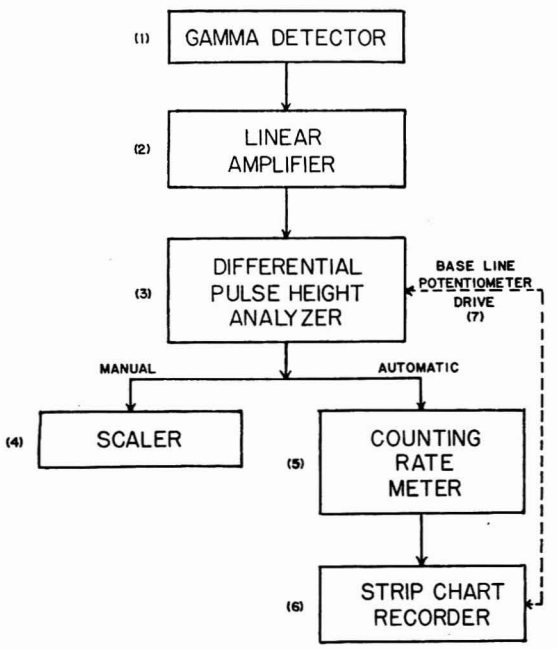


Figure 1. Block diagram of gamma scintillation spectrometer

Scintillation Spectrometer. The detector of the scintillation spectrometer consisted of a sodium iodide (Tl) crystal (Harshaw Chemical Co.) 1.5 inches in diameter and 0.5 inch deep viewed by a DuMont 6292 photomultiplier tube. A cathode follower coupled the output signal into a linear amplifier (Atomic Instrument Co.) which operated into a single-channel differential pulse height analyzer (Atomic Instrument Co.). The analyzer functions by accepting only those pulses within a narrow range of amplitudes called the slit width. This acceptance slit was made to scan the entire region of pulse amplitude by rotating the pulse analyzer bias potentiometer. The output pulses from the pulse height analyzer operated a counting rate meter (Nuclear Instrument & Chemical Co.), the output of which was plotted against the analyzer bias voltage by a strip chart recording potentiometer. For higher precision measurement the pulse height analyzer was operated into a scaler to measure the number of radioactive disintegrations per unit time. A block diagram of the spectrometer is shown in Figure 1 and the instrument itself is shown in Figure 2.

The peaked responses in the pulse height scan are known as photoelectric peaks and each photopeak occurs at a pulse height proportional to the incident gamma energy. In general, the maximum photopeaks were measured and qualitative identification of gamma emitters was made possible by comparing the voltage of the photopeak with a calibration curve for the instrument previously prepared using standard radioisotopes whose γ energies are well known (cobalt-60, antimony-125).

Quantitative estimation of the various radionuclides was accomplished by measuring the areas under the respective photopeaks and comparing them with the areas under the photopeaks obtained from the standards of known concentration. In all cases appropriate decay corrections were applied in calculating the quantities of trace impurities present. Since the shape of photopeaks approximates a normal curve of error, simplification in the measurement of areas was possible. The area was measured by using the following equation:

$$A = Sh_{max}\rho$$

where h_{max} = maximum photopeak height in counts per minute
ρ = width at half-maximum in units of slit width
S = 1.07 and is derived from a table of the normal curve of error

RESULTS AND DISCUSSION

Only those elements which result in radionuclides of half lives approximately greater than 2 hours and less than 200 days could easily be determined under the conditions of this experiment. This interval was chosen as most convenient for measurement since analysis of the samples started approximately 4 hours after removal from the pile. Radionuclides with half lives much shorter than 2 hours could not be detected conveniently in this laboratory when present in trace amounts, whereas radionuclides with half lives much greater than 200 days would be insufficiently activated for measurement under the conditions of the irradiation employed. There are, of course, exceptions to this general classification depending upon the nuclear properties of certain impurity elements. Thus, if the corresponding stable isotopes of these impurity elements possess optimum cross sections and abundances, the activity produced on irradiation

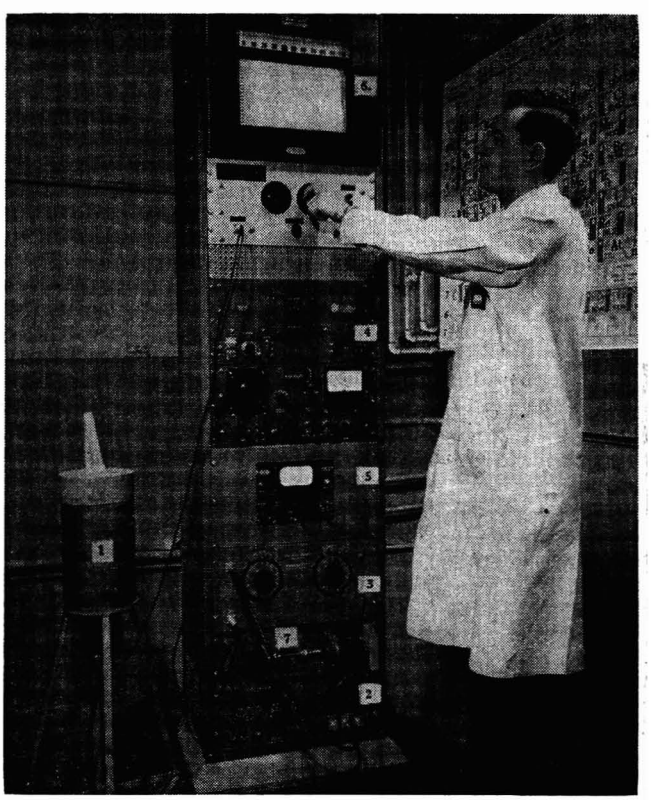


Figure 2. Gamma scintillation spectrometer

Components numbered according to diagram in Figure 1

may be sufficiently high for detection beyond the limits of the half lives mentioned above. Also, if the impurities are present in larger amounts, greater activity will result. This method was designed to determine as many elements as possible in a single irradiation, and those elements which can conveniently be determined in trace amounts by this method are shown in Figure 3.

Among the elements which, upon irradiation under the conditions used in this method, produce pure β emitters with half lives included in the chosen interval are calcium, yttrium, silicon, phosphorus, sulfur, and tellurium. Since they would not be detected by γ scintillation spectrometry, it was necessary to make composite β decay measurements and absorption measurements with calibrated aluminum absorbers, using Geiger counting techniques. These measurements also provide confirmative information for those isotopes which emit both β particles and γ photons.

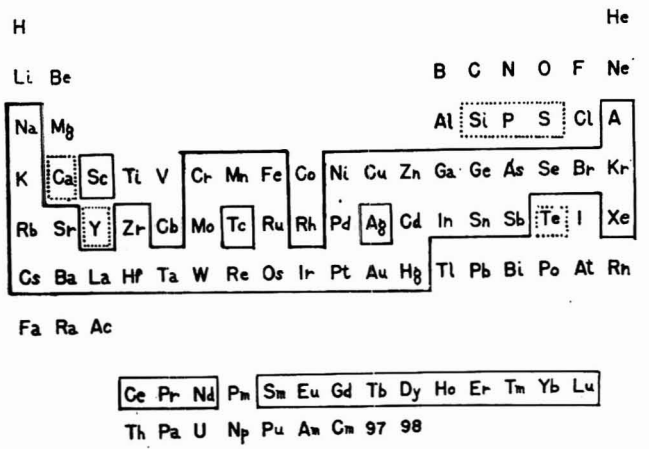


Figure 3. Trace elements producing measurable radioactivity

Based on 3-day irradiation at a flux of 3.4×10^{12} neutrons per sq. cm. per second and measurement of the short half-lived isotopes within 4 to 6 hours after removal from the pile. Elements in solid blocks form gamma emitting radionuclides measurable on scintillation spectrometer. Elements in broken blocks form only beta emitting radionuclides

The usefulness of the γ scintillation spectrometer in identifying those radionuclides produced by activation is based on the observation that the photopeak energy of the γ-emitting radionuclide may uniquely identify it or limit its identity to a few possibilities. A practical criterion for the resolution and identification of γ emitters is that the energies of the principal γ photons of the possible components differ by more than the half-maximum width (ρ) of the instrument. Many radionuclides produce more than one photopeak and consequently, in those cases where a photopeak may be attributed to several radionuclides, it is often possible to make positive identification by measurement of one of the auxiliary photopeaks. Also, scans of the γ spectra of the samples can be repeated at various intervals of time and the decay of the isotope obtained from the decrease in the area of the respective photopeaks. The value of the half life of the isotope obtained in this manner can then be used to confirm the presence of a single radionuclide. In cases where a particular combination of radionuclides cannot successfully be resolved by the spectrometer, it is necessary to resort to radiochemical separations using carriers.

The results of the analysis of the various silicon samples are given in Table II. Identification of the impurities was based on the energies of the gamma photons as measured by the spectrometer, as well as the half lives.

Accuracy and Precision. In an attempt to determine the precision of measurement of trace amounts of impurities by this method, an aluminum sample was analyzed for copper at four different concentrations, using the same number of counts per determination. An estimated standard deviation of 1.24% was obtained as shown in Table III.

Ten measurements of chromium in the same aluminum sample were made at one concentration and found to result in a standard deviation of 1.1%. Of this observed error the major portion, 1.0%, can be accounted for by the expected statistical fluctuation of radioactive decay. The error of the method, therefore, appears to be determined chiefly by the statistical fluctuation of counting, with very little contribution due to errors of manipulation. This counting error can be minimized by taking a sufficiently large count.

Table II. Determination of Impurities in Silicon

Sample[a]	Impurity	Radionuclide	Measured Gamma Energy, M.e.v.[b]	Measured Half Life[b]	Concentration, %
1	Zn	Zn^{69}	0.44	14h	2.2×10^{-2}
	As	As^{76}	0.55	25h	1.8×10^{-4}
	W	W^{187}	0.69	24h	5.5×10^{-5}
	Fe	Fe^{59}	1.09	44d	2.0×10^{-3}
	Na	Na^{24}	1.36	15h	8.5×10^{-6}
	K	K^{42}	1.52	12.4h	8.5×10^{-5}
2	Zn	Zn^{69}	0.44	14h	4.0×10^{-5}
	As	As^{76}	0.55	26h	1.6×10^{-2}
3	Zn	Zn^{69}	0.44	14h	6.0×10^{-3}
	As	As^{76}	0.55	26h	1.2×10^{-3}
4	As	As^{76}	0.55	26h	2.0×10^{-3}
	Ta	Ta^{182}	1.22	109d	8.3×10^{-4}
5	Zn	Zn^{69}	0.44	14h	7.4×10^{-2}[c]
	As	As^{76}	0.55	26h	2.2×10^{-4}[c]

[a] Refer to section on samples for description.
[b] Measured values for energies of gamma photons, and half lives agree to within 1.5 and 3.7%, respectively, of values reported by Hollander, Perlman, and Seaborg (*3*).
[c] Impurities found in 1-cm. end portion of single crystal 6.5 cm. long.

To determine the accuracy of the method for trace impurities, a National Bureau of Standards aluminum alloy (86-C) was analyzed for copper and chromium. The results are included in Table IV. These data indicate that the results agree very well with the values reported by the National Bureau of Standards. These results are valid only when the standard sample of the impurity being determined is irradiated with the unknown.

Table III. Effect of Concentration on Determination of Trace Amounts of Copper in Aluminum

Sample	Cu Measured, Gram	Cu in Al, %
1	2.04×10^{-5}	0.296
2	2.00×10^{-6}	0.290
3	2.01×10^{-7}	0.292
4	2.06×10^{-8}	0.298
		Av. 0.294 ± 0.004 (1.24%)

Table IV. Copper and Chromium in Aluminum Alloy 86-C

Element	Reported by NBS, %[a]	Found, %[b]	Impurity Measured, Gram
Cu	7.92 ± 0.03	8.01 ± 0.11	1.08×10^{-5}
Cr	0.029 ± 0.003	0.029 ± 0.0004	2.54×10^{-6}

[a] Sample means of 7 determinations for copper and 9 for chromium based on a number of different chemical methods.
[b] Analysis was performed on aliquots of sample after irradiation without chemical separations.

CONCLUSIONS

A method has been developed for the quantitative determination of many trace impurities in a single analysis with little or no chemical manipulation, thereby minimizing the possibility of loss or contamination. The method has been applied successfully to the analysis of trace impurities in silicon and aluminum; but it can easily be applied to many other materials. In those

cases where γ activity of the matrix interferes in the γ spectrometric analysis of the sample, and where the half life is not short enough to permit rapid decay of the interference, chemical separation can be employed. The method possesses a sensitivity of from 0.001 to 1 γ for the majority of the elements and exhibits good accuracy and precision in this trace range.

ACKNOWLEDGMENT

Acknowledgment is gratefully given to E. R. Bowerman for his many valuable suggestions and criticisms. The authors also wish to thank P. H. Keck of the Squier Signal Corps Laboratory for furnishing the single crystal of silicon grown by the floating zone technique.

LITERATURE CITED

(1) Boyd, G. E., ANAL. CHEM., **21**, 335 (1949).
(2) Connally, R. E., and Leboeuf, M. B., *Ibid.*, **25**, 1095 (1953).
(3) Hollander, J. M., Perlman, I., and Seaborg, G. T., *Revs. Mod. Phys.*, **25**, 469 (1953).
(4) Keck, P. H., Van Horn, W., Soled, J., and MacDonald, A., *Rev. Sci. Instr.*, **25**, 331 (1954).
(5) Leddicotte, G. W., and Reynolds, S. A., *Nucleonics*, **8**, 63 (1951).
(6) Litton, F. B., and Andersen, H. C., *J. Electrochem. Soc.*, **101**, 287 (1954).
(7) Lyon, D. W., Olson, C. M., and Lewis, E. D., *Ibid.*, **96**, 359 (1949).
(8) Lyon, W. S., and Manning, J. J., *Phys. Rev.*, **93**, 501 (1954).
(9) Taylor, T. I., and Havens, W. W., *Nucleonics*, **6**, 54 (1950).

RECEIVED for review November 27, 1954. Accepted January 21, 1955.

Optical-Analytical Studies on Steroids

Reducing Characteristics of Hydroxylated and Ketonic Steroids toward Blue Tetrazolium

ANDRE S. MEYER and MARJORIE C. LINDBERG

Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

The need for implementation of micromethods leading to an assignment of recognized functional groupings in complex molecules to their correct position instigated an investigation of the reducing characteristics toward blue tetrazolium of a number of Δ^4-3-ketosteroids with a hydroxyl or a keto grouping in various positions of the molecule. These functions, dependent on their location and configuration, gave rise to varied rates and intensities of color formation. The complex nature of reaction of the Δ^4-3-ketosteroids limited the diagnostic value of those rate measurements for structural characterization on a microlevel to certain well-studied examples. The conversion of Δ^4-androstene-3,17-dione to Δ^4-androsten-6β-ol-3,17-dione and Δ^4-androstene-3,6,-17-trione by alkaline blue tetrazolium has been demonstrated.

THE increasing importance of tetrazolium salts as hydrogen acceptors in biological redox reactions with the subsequent formation of deeply colored formazans has stimulated the preparation of new members of this class (for reviews, see *1*, *15–19*). Some of these compounds have been introduced recently in steroid chemistry for the qualitative demonstration (*4*) and the quantitative estimation (*5*, *8*, *10*, *13*) of corticosteroids. The observation that the non-α-ketolic 6α-hydroxy-Δ^4-androstene-3,17-dione (*2*) exhibited, when tested on paper (*12*), a retarded formazan formation with the blue tetrazolium [2,2′-*p*-(di-*o*-methoxy)-diphenylene-3,3′5,5′-tetraphenylditetrazolium chloride] (*16–18*) reagent, whereas the analogous 6β-hydroxyl derivative did not, instigated the present investigation of a number of biologically important Δ^4-3-ketosteroids (parent compounds) and their hydroxylated derivatives with respect to their reducing characteristics (Tables I and II and Figure 1). This was done in order to determine whether a correlation between the color production and the location of the structural function in the polycyclic nucleus could be established. This report deals with the results of such a study.

RESULTS

The steroids were exposed to blue tetrazolium in ethanolic solution at various alkalinities and temperatures, and the color development was measured at appropriate intervals of time. The observed absorbance (equal to the negative logarithm of the transmittance) was, for comparison purposes, referred to 0.040 micromole of substance. Steroids with a primary α-ketol group reduced blue tetrazolium more readily than those formazan-producing compounds without that grouping and were thus easily distinguishable. Representative data for one compound of each series, investigated under a variety of conditions, are listed in Table III. Deoxycorticosterone (2) reached maximum intensity of its chromogen in all instances within 45 minutes ($\epsilon \sim 23 \times 10^3$), whereas the slower formazan formation of 6α-hydroxy-Δ^4-androstene-3,17-dione (1) was a function of the alkalinity and the temperature. Not only the rate of color development but also its maximum intensity (reproducibility $\pm 5\%$) was dependent on these conditions. The range of basicity was achieved by the use of three tetraalkylammonium hydroxides. Tetramethylammonium hydroxide (TMAH) had the one disadvantage that the color formed was destroyed to a larger degree, over the extensive reaction period or at the elevated alkalinity required, than when triton B (R_1 = benzyl) or choline (R_1 = β-hydroxyethyl) or approximately equimolar solutions of these bases with tetramethylammonium hydroxide were used. The less reproducible destruction of the formazan (*9*, *15*) did not seriously impair the reaction, since the concurrent production of the chromogen was usually predominant. Although a reaction period of 2 to 3 hours—e.g., conditions V and XII—could be considered suitable for general application, it may in certain cases be desirable to preserve valuable characteristics of the color formation as can be observed under less vigorous conditions in an early stage—e.g., III and IX. As an expedient, the reaction can be started at room temperature at an elevated basicity and, after a few readings, terminated at a higher temperature, thus keeping the reaction time within practical limits—e.g., conditions XI. With a more stable formazan compound it should be possible to carry out the reaction at higher alkalinities and room temperature in a shorter period of time.

The chromogen formation by simple Δ^4-3-ketosteroids with various substituents at carbon-17 (under conditions IX) is illustrated in Figure 2. Changed conditions influenced the shape of the curves to some extent, although the order of magnitude was in most cases maintained. Similar time curves to that of compound 3 were recorded for 11α-hydroxy-Δ^4-androstene-3,17-dione (11), to that of compound 4 for 11α-hydroxy (12), 16α-hydroxy (13), and 17α-hydroxyprogesterone (14), and to that of

Table I. Compounds Investigated

[Nomenclature according to (6)]

No.	Compound	No.	Compound
1	6α-Hydroxy-Δ⁴-androstene-3,17-dione and acetate	22	Androstane-3,6,17-trione
2	11-Deoxycorticosterone	23	7β-Hydroxy-Δ⁴-cholesten-3-one
3	Δ⁴-Androstene-3,17-dione	24	2α-Hydroxytestosterone
4	Progesterone (Δ⁴-pregnene-3,20-dione)	25	19-Hydroxy-3-keto-Δ⁴-etienic acid
5	Δ⁴-Androsten-3-one	26	19-Nor-Δ⁴-androstene-3,17-dione
6	Testosterone (Δ⁴-androsten-17β-ol-3-one)	27	Δ⁴-Androstene-3,11,17-trione
7	Δ⁴-Cholesten-3-one	28	6α-Hydroxyprogesterone
8	3-Keto-Δ⁴-etienic acid	29	6α-Acetoxy-17α-hydroxyprogesterone
9	Δ¹,⁴-Androstadiene-3,17-dione	30	6α-Acetoxy-Δ⁴-cholesten-3-one
10	Δ¹,⁴-Androstadiene-3,17-dione	31	6α-Acetoxy-deoxycorticosterone acetate
11	11α-Hydroxy-Δ⁴-androstene-3,17-dione	32	6α-Acetoxy-17α-hydroxydeoxycorticosterone acetate
12	11α-Hydroxyprogesterone		
13	16α-Hydroxyprogesterone	33	19-Norprogesterone
14	17α-Hydroxyprogesterone	34	19-Hydroxyprogesterone
15	11α-Hydroxy-3-keto-Δ⁴-etienic acid	35	2α-Acetoxyprogesterone
16	16α-Hydroxy-Δ⁴-androstene-3,17-dione	36	11-Ketoprogesterone
17	19-Hydroxy-Δ⁴-androstene-3,17-dione	37	Cortisone (17α-hydroxy-11-dehydrocorticosterone)
18	14ξ-Hydroxy-Δ⁴-androstene-3,17-dione	38	Aldosterone (18-oxocorticosterone)
19	12α-Hydroxy-Δ⁴-androstene-3,17-dione	39	Δ⁵-Androstene-3β,17β-diol-16-one
20	11β-Hydroxy-Δ⁴-androstene-3,17-dione	40	Cortisone-21-aldehyde
21	6β-Hydroxy-Δ⁴-androstene-3,17-dione and acetate	41	1,4-Cyclohexanedione
		42	Hydroquinone (1,4-benzenediol)

Table II. Compounds with No Significant Reaction toward Alkaline Blue Tetrazolium (under Conditions XII)

[Nomenclature according to (3, 6, 14)]

No.	Compound	No.	Compound
43	Androstane-3,17-dione	51	Cholestan-3-one enol acetate
44	Androstan-3α-ol-17-one (androsterone)	52	Estrone
45	Androstan-3β-ol-17-one (epiandrosterone)	53	Solanidine
46	Δ⁵-Androsten-3β-ol-17-one (dehydroepiandrosterone)	54	Isorubijervine
47	Δ⁵-Androstene-3α,17β-diol	55	Methyl hederagonate
48	Δ⁴-Androstene-3β,17β-diol	56	Icterogenin
49	Δ¹-Androstene	57	5,5-Dimethyl-1,3-cyclohexanedione (dimedone)
50	Δ⁴-Cholestene-3,6-dione	58	Formaldehyde

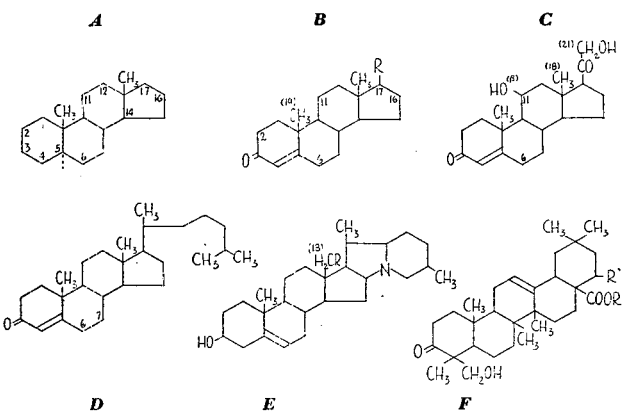


Figure 1. Types of structures investigated

A. **Androstane**
B. **Progesterone (4), R = $COCH_3$**
3-Keto-Δ⁴-etienic acid (8), R = COOH
C. **Corticosterone**
D. **Δ⁴-Cholesten-3-one (7)**
E. **Solanidine (53), R = H; Isorubijervine (54), R = OH**
F. **Methyl hederagonate (55), R = CH_3, R′ = H**
Icterogenin (56), R = H, R′ = O.COC_4H_7

compound 8 for 11α-hydroxy-3-keto-Δ⁴-etiocholenic acid (15). Substances found to produce practically no color are listed in Table II. Among the tested solvents, purified ethyl alcohol was found to give relatively smallest blank readings (Figure 2).

The hydroxylated Δ⁴-androstenedione derivatives studied showed remarkably different curves (Figure 3). According to its position and configuration, the hydroxyl group may inhibit the chromogen formation of its parent compound (20 and 21), influence it little (19 and other examples mentioned above), or make an additional contribution to the color production as in compounds 1, 16, 17, and 18. The acetates of compounds 1 and 21, on comparison with the respective free substances, resulted in the same time curves. Compound 1 obeyed Beer's law at steroid concentrations from 2 to 8 × $10^{-5}M$. It is remarkable that androstane-3, 6,17-trione (22) showed under conditions III approximately the same rate of formazan formation as was observed with 6α-hydroxyandrostenedione (1) (Figure 3). *Furthermore,* the 11-keto group in the androstenedione molecule (27) contributed a notable color (Figure 4). As only a limited number of androstenedione derivatives hydroxylated in various positions were known, the study was completed with hydroxylated derivatives of other Δ⁴-3-ketosteroids. To facilitate a comparison, curves were plotted for the formular absorbance increment of these steroids over their parent compounds determined simultaneously—e.g., the difference in absorbance of compound 1 and of 3 was calculated for each reading (Figure 4). The increment for 7β-hydroxy-Δ⁴-cholestenone (23) was high, nearly twice as large as that found for 2α-hydroxytestosterone (24). Removal of the angular methyl group at carbon-10 (19-norandrostenedione) (26) also gave rise to an intensified color formation.

The increment rates have been found to be similar for various molecules with a particular structural function. A close correspondence was found for the rates of the increase in color production of the 6α-hydroxyl derivatives of androstenedione (1) and progesterone (28), and the 6α-acetates of 17α-hydroxyprogesterone (29) and cholestenone (30). In good agreement with these were the analogous curves for the diacetates of 6α-hydroxydeoxycorticosterone (31) and 6α-hydroxy-17α-hydroxydeoxycorticosterone (32) when the relatively appreciable destruction of the chromogen of the parent compound was taken into account. The above measurements were made under conditions III and IX; conditions VII were less favorable for such a comparison. A correlation was also found with 19-norprogesterone (33) and 19-norandrostenedione (26). Again 19-hydroxypro-

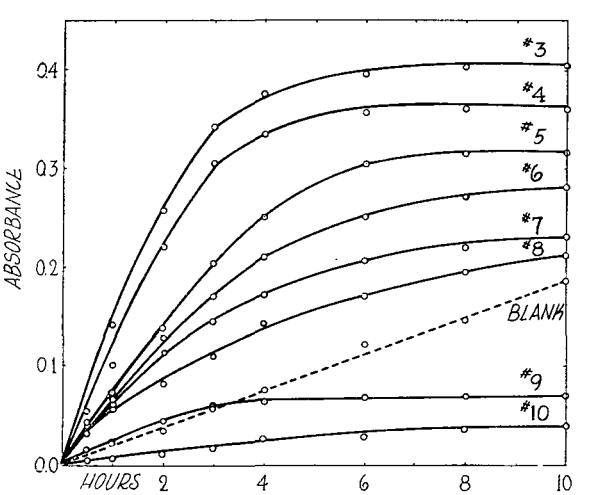


Figure 2. Rate of blue formazan formation for 0.040 micromole of various Δ⁴ -3-ketosteroids under conditions IX

Curve numbers correspond to compound numbers listed in Table I. Absorbance of reaction blank read against ethyl alcohol

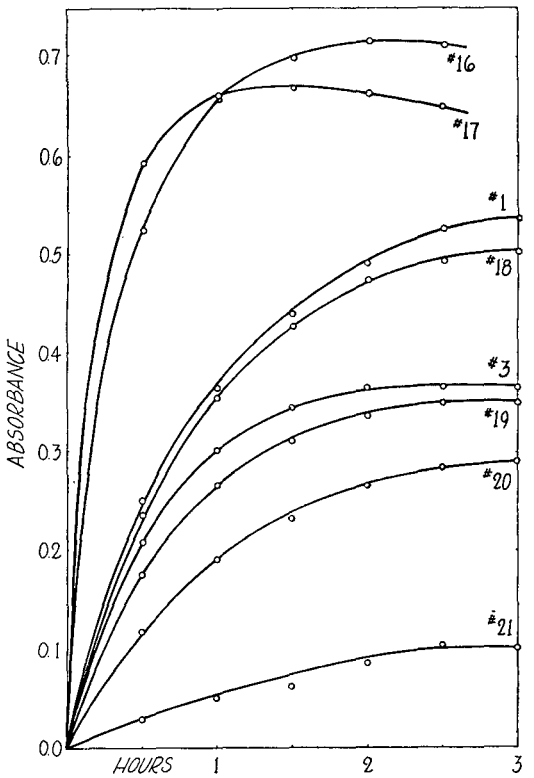


Figure 3. Rate of blue formazan formation for 0.040 micromole of hydroxylated Δ^4-androstene-3,17-dione derivatives under conditions XII

Table III. Blue Formazan Formation under Various Conditions

Condition No.	Base	Normality	Temp., ° C.	Maximum Color Formation, Absorbance per 0.040 Micromole: 6α-Hydroxyandrostenedione (1) Hours	6α-Hydroxyandrostenedione (1) Absorbance	Deoxycorticosterone (2), after 45 min.
I	TMAH	0.0055	37	30	0.71	0.77
II	TMAH	0.017	25	20	0.61	0.60
III	TMAH	0.017	37	8	0.60	0.71
IV	TMAH	0.028	37	4	0.56	0.68
V	TMAH	0.055	37	2.5	0.41	0.65
VI	Choline	0.066	37	3.5	0.45	0.69
VII	Triton B	0.060	37	3.5	0.48	0.68
VIII	Triton B	0.060	45	2.5	0.57	0.82
IX	TMAH	0.008	37	8	0.62	0.74
	Choline	0.007				
X	TMAH	0.008	50	4	0.60	0.76
	Choline	0.007				
XI[a]	TMAH	0.016	25	1.5	0.60	0.74
	Choline	0.014	50	1.5		
XII	TMAH	0.028	37	3	0.53	0.72
	Choline	0.022				

[a] Color developed by exposure first to 25° C. for 1.5 hours and then to 50° C.

gesterone (34) and 19-hydroxyandrostenedione (17) were comparable in their chromogens, and 19-hydroxy-3-keto-Δ^4-etienic acid (25) developed an increment of the same maximum intensity, however, at a slower rate (Figure 4). Furthermore, under conditions XII the increments of 2α-hydroxytestosterone (24) and 2α-acetoxyprogesterone (35) agreed well. Adrenosterone (27) and 11-ketoprogesterone (36) showed a similar increase in color formation over their parent compounds, whereas that of cortisone (37) appeared to be less. Analogous comparisons should be possible with other polycyclic systems, since the tested steroidal alkaloids (compounds 53 and 54) and triterpenes (compounds 55 and 56) without conjugated ketonic grouping did not react with alkaline blue tetrazolium (Table II).

It was confirmed that several pure corticosteroids and their acetates produced essentially the same chromogen as did deoxycorticosterone (2). Comparable values were further obtained in 45 minutes of reaction time with 18-oxocorticosterone (aldosterone) (38) under conditions IX and with the cyclic secondary α-ketolsteroid Δ^5-androstene-3β,17β-diol-16-one (39) under conditions III. The delayed formazan formation of the two other types of secondary α-ketolsteroids examined (16, a 17,16α-ketol and 24, a Δ^4-3,2α-ketol) has been mentioned. The pronounced difference in the reducing characteristics of the 16,17β-ketol and the 17,16α-ketol is noteworthy. Cortisone aldehyde (Δ^4-pregnen-21-oxo-17α-ol-3,11,20-trione) (40) led to a gradual formation of

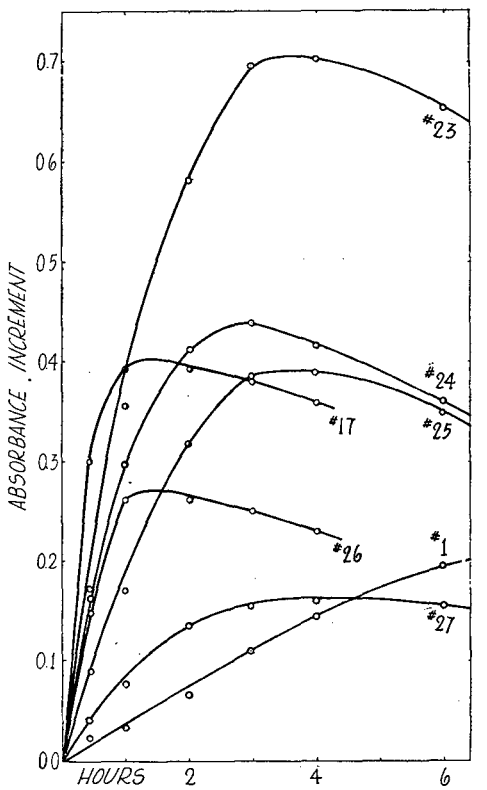


Figure 4. Increment of blue formazan formation rate for 0.040 micromole of oxygenated or 19-nor-Δ^4-3-ketosteroids under conditions IX

Color formation of parent compounds, see Figure 1

the formazan which eventually amounted to one third that of the standard deoxycorticosterone. 1,4-Cyclohexanedione (41), however, gave after 45 minutes a chromogen of the magnitude of the standard (under conditions I, a somewhat higher and under conditions XII, a somewhat lower value), and hydroquinone (42) showed (in both instances) half the intensity of the latter, whereas dimedone (57) did not react.

METHOD

Commercially available steroids were purified by direct crystallization or after chromatography, while other compounds were prepared according to published procedures.

The 95% ethyl alcohol (C.P.) was tested for its reducibility of blue tetrazolium by running a reaction blank. Some lots of distilled ethyl alcohol could be used as such; other batches produced too high blank readings and had to be refluxed over alkaline blue tetrazolium, and redistilled. In cases where the reaction blanks were still high, a prior refluxing over finely ground hydrated ferrous sulfate for 24 hours was found advantageous. The purified ethyl alcohol was stored under nitrogen in a dark bottle.

The alkaline ethyl alcohol reagent was freshly prepared as follows: For conditions III (Table III), 6 ml. of 10% (~1.1*N*)

aqueous tetramethylammonium hydroxide were mixed with 94 ml. of the purified ethyl alcohol and, if necessary, filtered under a nitrogen atmosphere; the reagent was thus 0.066N in the hydroxide. Because, in the course of the reaction, it was diluted to four times its volume, the final alkalinity (indicated in Table III) was 0.0165N. Choline (~2.6N) and triton B (~2.0N) were employed as 30% aqueous solutions. The final ethyl alcohol content of the medium ranged thus between 92 to 94%, varying for the conditions employed.

An aliquot of 0.50 ml. containing a known quantity between 10 to 16 γ of steroid (~0.045 micromole) in purified ethyl alcohol was pipetted into a glass-stoppered 10 × 75 mm. Coleman cuvette, and 0.25 ml. of a 0.1% (weight/volume) ethanolic solution of recrystallized blue tetrazolium (~0.35 micromole) was added. Finally, 0.25 ml. of the alkaline reagent was added and the solution was immediately mixed by inverting the stoppered cuvette twice. Each steroid was determined in duplicate. In each series of measurement deoxycorticosterone was run as standard of reference. The rack holding the series of up to 30 cuvettes was then placed in a light-free, thermostatically controlled cabinet at the desired temperature. The absorbance was read at appropriate time intervals against a reaction blank in a Coleman Junior spectrophotometer at 530 mμ at room temperature (25° C.), requiring 5 to 10 minutes for a series. The duplicates were generally found to differ by not more than 0.02 in absorbance. The mean value of the duplicates was recorded, referred to 0.040 micromole and plotted with respect to time.

DISCUSSION

This investigation can perhaps be best related to an earlier study of Heard and Sobel (*7*) in which the reducing power of various steroids was measured by means of the color development when the substances were heated at 100° C. for 20 minutes to 3 hours with a phosphomolybdic acid reagent in an acetic acid medium. Many similarities in the reducing characteristics of the various steroid classes toward both reagents were discerned. The principal distinction consisted in the fact that steroids reacted with blue tetrazolium under more gentle conditions, and those with a primary α-ketol grouping formed formazan chromogens of comparable intensities (*21*). In contrast, the molybdenum blue development by various side chain ketolic steroids differed considerably—e.g., cortisone produced a chromogen of only 70% of that of deoxycorticosterone. The data on the saturated steroids with a cyclic secondary α-ketol grouping at carbon-11,12, carbon-12,11(α and β), and carbon-3,2(α and β), of which these workers studied one example of each type, indicated that the molybdenum blue reached an absorptivity of the magnitude of 45% of deoxycorticosterone.

The blue tetrazolium reagent has found extensive application for the quantitative estimation of corticosteroids following methods described by Chen and coworkers (*5*) and Mader and Buck (*10*). Under the proper conditions the presence of moderate amounts of simple Δ^4-3-ketosteroids does not interfere with the measurement of the corticosteroids (*5, 10*). The gathered data revealed that at minimum basicity—e.g., 0.0025N in tetramethylammonium hydroxide or conditions I—relative short reaction time (45 and 15 minutes, respectively), and a temperature of about 40° C., the specificity of blue tetrazolium toward primary α-ketols is the greatest.

The corticosteroids in this investigation were used for reference only. That certain hydroxyl and ketone groups in the steroid molecule demonstrate considerable reducing power toward blue tetrazolium is noteworthy (Figures 3 and 4). (In paper chromatography, many of these compounds developed the purple color when the strip, after being sprayed with alkaline blue tetrazolium, was exposed to a stream of warm air.) According to their position and configuration in the molecule, remarkable differences in the reducing characteristics exist which may be used as a diagnostic means for the location of these functions in unknown compounds. Using conventional instrumentation, only 20 γ of the pure substance are required. The specificity of the changes in rate due to a particular structural grouping could not be established by this investigation of limited scope. Many more compounds would have to be examined and it is, for the time being, impossible to derive from these data alone definite characterization of unknowns. Moreover, possible interactions between the grouping under consideration and the parent substance should not be lost sight of when dealing with new structures. However, in specific cases where alternate possibilities are in question, the formazan rates may allow a reasonable choice. Two applications of this method have been described (*11, 12*). Some discrepancies in increment rates for the same function have been noted, which limits the general applicability of this procedure.

Two applications (12) of this method with presumably hydroxylated Δ^4-androstene-3,17-dione derivatives may exemplify its usefulness. One of these unknown compounds, isolated in small quantities, showed a similar, although somewhat decreased formazan formation than did 6α-hydroxyandrostenedione (1). Infrared analyses indicated that another hydroxyl group was attached at C-11β. Since a hydroxyl group at that position was shown to inhibit the chromogen formation of the parent Δ^4-3-ketosteroid, the correlation of the curves improved. Finally, the characteristic light absorption in alkaline ethanol for a Δ^4-3-keto-6α-hydroxyl structure (11) was noted, and these data permitted the tentative conclusion that this unknown sample was the 6α,11β-dihydroxy derivative. Another isolated compound demonstrated an unusual rate of chromogen formation which allowed the exclusion of all the structural possibilities previously examined.

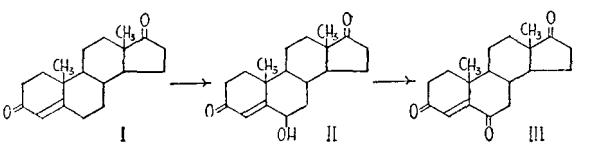


Figure 5. Transformation products

Various isolated carbonyl, hydroxyl (primary and secondary), or enol groupings or olefinic linkages, as well as α,β- or β,γ-unsaturated hydroxyl groups did not induce a dehydrogenation in the steroid molecule by the blue tetrazolium reagent (Table II and compounds 11 to 15). However, the Δ^4-en-3-one system gave rise to ample formazan formation, in contrast to the limited ability of the $\Delta^{1,4}$-dien-3-one (10) and $\Delta^{4,6}$-dien-3-one (9) structures (Figure 2). In the case of Δ^4-androstene-3,17-dione (I) a conversion to 6β-hydroxyandrostenedione (II) and 6-ketoandrostenedione (III) was ascertained through isolation of these transformation products (Figure 5). This oxidation appeared to be influenced to an unexpectedly large extent by functional groupings quite distant from the conjugated ketonic system. For example, the different side chains at carbon-17 affected the color development, and the 11β-hydroxyl group (20), which is situated in γ-position to the conjugated double bond in ring A, distinctly suppressed the reaction. The 11α-hydroxyl group (11, 12, and 15), however, had no influence on the chromogen, whereas the presence of the 11-keto group (27 and 36) enhanced it. The tertiary hydroxyl group at carbon-14 (18) added significantly to the color. That the 6β-hydroxyl group (21) decreased the formazan production is explained by the demonstrated course of reaction. It would appear that the 6α-hydroxy-Δ^4-3-keto system (1) was isomerized in the alkaline medium to the 3,6-diketo 5α-configuration (22) or a common enolate, as both substances showed approximately the same rate of formazan formation. In another γ-diketone, 1,4-cyclohexanedione (41), an oxidation to the quinone appeared to occur, as hydroquinone (42) yielded only half the color intensity.

The 19-norsteroids (26 and 33) produced considerably more color than their corresponding 10-methylated compounds. This was due to the more complex alterations these compounds underwent, as was seen in an experiment on a larger scale with 19-

norandrostenedione. Some products showed typical aromatic properties when examined spectrometrically, indicating a conversion to estrone-type steroids. Such a course of reaction might possibly also apply to the 19-hydroxylated Δ^4-3-ketosteroids (17 and 34), as their transformation in alkaline medium to 19-nor compounds has been demonstrated (*11*) and the formazan increments reached their maximum in both cases after 1.5 hours (Figure 4). Ultraviolet measurements indicated that the 7β-hydroxy-Δ^4-cholesten-3-one (23) was dehydrated in alkaline medium (in absence of a tetrazolium salt) to the $\Delta^{4,6}$-dien-3-one derivative (9) (unpublished observation). In the presence of blue tetrazolium such dehydration was not favored, as compound 9 reacted only very slowly, whereas with compound 23 an efficient dehydrogenation took place. It has been demonstrated that the secondary α-ketol 2-hydroxy-1-cyclohexanone is oxidized with triphenyltetrazolium chloride to the corresponding dione (*20*). An analogous reaction can be assumed as having occurred on the ketol moiety of compounds 16 and 24. Deoxycorticosterone and its 17α-hydroxyl analog have been degraded by blue tetrazolium to their corresponding 17-carboxylic acids, as shown by the isolation of 3-keto-Δ^4-etienic acid and 3-keto-17α-hydroxy-Δ^4-etienic acid (*21*). Formaldehyde (58) which conceivably could arise from the breakdown of this side chain, would not be further oxidized by blue tetrazolium, as it did not give any color with the reagent. A similar course of reaction as with the 20,21-ketols seemed to be indicated for the 16,17β-ketol (39); in the latter case a fission of ring D would be involved.

EXPERIMENTAL

Conversion of Δ^4-Androstene-3,17-dione (I) to 6β-Hydroxyandrostenedione (II) and 6-Ketoandrostenedione (III). Chromatographically pure Δ^4-androstene-3,17-dione (I) (26.3 mg.) and blue tetrazolium (170 mg.) were dissolved in purified ethyl alcohol (155 ml.), and were made alkaline with a 50% aqueous choline solution (0.80 ml.) and a 10% aqueous tetramethylammonium hydroxide solution (3.95 ml.). The batch was heated in the dark for 3 hours at 37° C. After that period, 95% of the mixture was cooled to room temperature and L-ascorbic acid (100 mg.) was added for reduction of unreacted tetrazolium salt. The mixture was then neutralized with 1*N* hydrochloric acid (7.4 ml.), and evaporated to complete dryness in vacuo below 40° C. under a nitrogen atmosphere. The residue was shaken with distilled water (500 ml.) at room temperature for 4 hours, when the undissolved formazan was quantitatively separated by suction through a Celite filter. The filter residue was shaken overnight with another portion (300 ml.) of water. To the combined aqueous filtrate sodium chloride (60 grams) was added, and the solution extracted in 60-ml. portions consecutively with three portions of ether (each 400 ml.). The ether extracts were washed with two portions of water (15 ml.), dried over anhydrous sodium sulfate, and evaporated in vacuo.

The residue (23 mg.) was applied in the usual manner (*4*, *12*) on two sheets of Whatman paper No. 1 (17 cm. wide) and chromatographed with propylene glycol–saturated ligroin at 25° C. for 30 hours. A minute quantity of unreacted I had been caught with the overflow. Scanning the chromatograms with ultraviolet light revealed the presence of two α,β-unsaturated ketonic products in larger concentrations.

The first zone located at 1 to 2.5 cm. from the origin was eluted and rechromatographed on a 6-cm. sheet in the toluene–propylene glycol system. The material yielded after recrystallization from acetone-ether, 2.5 mg. of slightly impure 6β-hydroxy-Δ^4-androstene-3,17-dione (II), melting point 192–195° C., $\lambda_{max.}^{C_2H_5OH}$ 236 mμ. The melting point was not depressed on admixture with a reference compound (*12*) and the infrared spectra of both samples were dentical.

The second zone at 23 to 29 cm. from the origin produced with 14% ethyl alcoholic potassium hydroxide a strong yellow color, characteristic of the Δ^4-3,6-diketo structure. Less intense coloration was exhibited on the paper in the adjoining zone at 15 to 23 cm. The eluate of this trailing zone was rechromatographed on a 7-cm. sheet for 3 hours ascendingly in the toluene–propylene glycol system in which the substance moved as a relatively narrow band with a R_f 0.5. The combined eluates yielded, after crystallization from ethyl acetate and acetone-ether, 8.5 mg. of Δ^4-androstene-3,6,17-trione (III), melting point 225–227° C.; $\lambda_{max}^{C_2H_5OH}$ 251 mμ (log ϵ 4.04), $\lambda_{max}^{C_2H_5OH\text{-}KOH}$ 259 mμ (log ϵ 4.03), 380 mμ (log ϵ 3.96) (data to be published); $\bar{\nu}_{min}^{solid}$ 1730 cm.$^{-1}$ (17-keto), 1686 cm.$^{-1}$ (Δ^4-3,6-diketo), 1612 cm.$^{-1}$ (conjugated olefine); the infrared spectrum was identical with that of a reference sample. With the *m*-dinitrobenzene reagent (*12*) a small quantity of a reduced 17-ketosteroid, which did not absorb the ultraviolet light, was located in a zone at 5 to 6 cm. from the origin.

An aliquot of 5% of the batch was withdrawn after 0.5 hour of reaction time and processed in a manner analogous to the above. According to evidence by chromatogram, the yields of II and III were of the same order of magnitude as in the experiment of longer duration, although much unreacted I was still present and the formazan absorbance amounted to only two fifths the value of the 3-hour run. It thus would appear that especially III was rather instable in the reaction medium.

A sample of the above 6β-hydroxyandrostenedione (II) (1.5 mg.) was reacted for 3 hours under conditions analogous to those for the androstenedione (I). The absorbance of the formazan formed was determined under the dilution used in the analytical measurements to give a value of 0.28 for 0.040 micromole of II. Of the recovered material, approximately one half had been converted to III as estimated by ultraviolet measurements of the eluates of the paper chromatogram.

ACKNOWLEDGMENT

This study was made possible by the generous contributions of those who kindly furnished the required samples. Thanks are given to D. H. R. Barton for compounds 55 and 56; to Maximilian Ehrenstein for all nine specimens oxygenated at carbon-6 (with the one listed exception) and for compounds 25 and 34; to L. F. Fieser for compounds 30 and 50; to Josef Fried, Squibb Institute for Medical Research, for compounds 13 and 16; to Marcel Gut for compounds 7, 10, 26, 47, and 48; to W. A. Jacobs for compounds 53 and 54; to D. H. Peterson, Upjohn Co., for compounds 11 and 36 and reference compound III; to Tadeus Reichstein for compound 38; to I. V. Sollins, Chemical Specialties Co., Inc., for compounds 9 and 33; to Franz Sondheimer, Syntex S. A., for compounds 24 and 35; to A. F. St. André, Ciba Pharmaceutical Products, Inc., for compounds 5 and 18; to Max Tishler, Merck & Co., Inc., for compounds 12 and 40; and to Albert Wettstein, Ciba, Ltd., for compound 19. The support of this investigation by grants from the National Institutes of Health (Research Grant G-3247) of the U. S. Public Health Service, and from G. D. Searle and Co., Chicago, Ill., is gratefully acknowledged.

LITERATURE CITED

(1) Ashley, J. N., Davis, B. H., Nineham, A. W., and Slack, R., *J. Chem. Soc.*, **1953**, 3881.
(2) Balant, C. P., and Ehrenstein, M., *J. Org. Chem.*, **17**, 1587 (1952).
(3) Barton, D. H. R., and deMayo, P., *J. Chem. Soc.*, **1954**, 887.
(4) Burton, R. B., Zaffaroni, A., and Keutmann, E. H., *J. Biol. Chem.*, **188**, 763 (1951).
(5) Chen, C., Wheeler, J., and Tewell, H. E., *J. Lab. Clin. Med.*, **42**, 749 (1953).
(6) Fieser, L. F., and Fieser, M., "Natural Products Related to Phenanthrene," **3rd** ed., Reinhold, New York, 1949.
(7) Heard, R. D. M., and Sobel, H., *J. Biol. Chem.*, **165**, 687 (1946).
(8) Henly, A. A., *Nature*, **169**, 877 (1952).
(9) Jambor, B., *Ibid.*, **173**, 774 (1954).
(10) Mader, W. J., and Buck, R. R., ANAL. CHEM., **24**, 666 (1952).
(11) Meyer, A. S., *Experentia*, **11**, 99 (1955).
(12) Meyer, A. S., Hayano, M., Lindberg, M. C., Gut, M., Rodgers, O. G., *Acta Endocrinol.*, **18**, 148 (1955).
(13) Oertel, G., *Ibid.*, **16**, 267 (1954).
(14) Pelletier, S. W., and Jacobs, W. A., *J. Am. Chem. Soc.*, **75**, 4442 (1953).
(15) Ried, W., *Angew. Chem.*, **64**, 391 (1952).
(16) Ried, W., and Gick, H., *Ann.*, **581**, 16 (1953).
(17) Rutenberg, A. M., Gofstein, R., and Seligman, A. M., *Cancer Research*, **10**, 113 (1950).
(18) Seiler, H., and Schmid, H., *Helv. Chim. Acta*, **37**, 1 (1954).
(19) Smith, F. E., *Science*, **113**, 751 (1951).
(20) Stoll, A., Stauffacher, D., and Seebeck, E., *Helv. Chim. Acta*, **36**, 2027 (1953).
(21) Zaffaroni, A., *Recent Progr. Hormone Research*, **8**, 51 (1953).

RECEIVED for review June 28, 1954. Accepted November 20, 1954.

Coulometric Determination of Selenium

KEITH ROWLEY and ERNEST H. SWIFT

California Institute of Technology, Pasadena 4, Calif.

The coulometric titration of thiosulfate with iodine has been applied to the determination of microgram quantities of selenious acid. Two methods utilizing reactions used in conventional volumetric determinations have been studied. Selenopentathionate is formed in one and elemental selenium in the other. The effect of oxygen has been investigated. The results obtained from the use of a simple modification of the Norris and Fay volumetric titration of selenium are also reported.

THE purpose of this work was to investigate the application of the coulometric titration of thiosulfate with electrolytically generated iodine (*14*) to the determination of microgram quantities of selenium. In preparation for the coulometric titrations a study was made of the modification suggested by McNulty (*6*) of the Coleman-McCroskey (*1*) volumetric procedure. The results of this volumetric study are presented and discussed first, as the same reactions are involved in the coulometric titrations.

VOLUMETRIC TITRATION OF SELENIOUS ACID

Two methods for the determination of selenious acid make use of standard thiosulfate solutions (*1, 3-6, 9-11, 15, 16*). In the first, a large excess of iodide is added, and the iodine is liberated by the reaction

$$4H^+ + 4I^- + H_2SeO_3 = Se + 2I_2 + 3H_2O \qquad (1)$$

and is titrated with standard thiosulfate (*3, 9, 19*). This method tends to give low results (*2, 9*) and especially with other than small quantities of selenium the starch end point is obscured by the red precipitate. In the second method, originated by Norris and Fay (*11*), an excess of standard thiosulfate is added to the selenious acid whereupon selenopentathionate and tetrathionate are formed by the following reaction

$$4H^+ + 4S_2O_3^{--} + H_2SeO_3 = Se(S_2O_3)_2^{--} + S_4O_6^{--} + 3H_2O \qquad (2)$$

The excess of thiosulfate is back-titrated with a standard iodine solution. This method has been shown by Coleman and McCroskey (*1*) to give results accurate to within 0.2% at room temperature if a large excess of standard thiosulfate is avoided. In both methods 1 mole of selenious acid is equivalent to 4 moles of thiosulfate.

McNulty (*6, 7*) has suggested a combination of these two methods in a direct titration. A small amount of potassium iodide is added to the selenious acid and a direct titration is made immediately to the starch end point with standard thiosulfate. Under these conditions the reaction of thiosulfate with selenious acid is faster than its reaction with iodine, therefore Reaction 2 predominates throughout the titration. If starch is present, the starch-iodine color which forms on addition of the iodide is visible throughout the titration; at the end point, when no significant quantity of selenious acid is left, the thiosulfate reduces the iodine. Under the conditions of the titration only a small amount of selenium is formed and the end point is not obscured. Although McNulty reduced the quantity of added iodide, it was more than equivalent to the selenium present. Thus, unless the titration were made immediately Reaction 1 could predominate; also, his data are inadequate to show the potential accuracy of his method, especially when it is used with larger quantities of selenium.

Accordingly, an investigation was made of the volumetric titration of macro quantities of selenium by two modifications of McNulty's method. In one of these, the selenious acid was titrated directly with thiosulfate to within a few per cent of the equivalence point before addition of iodide. In the second procedure, the equivalents of iodide added before beginning the titration were small compared with the equivalents of selenium present. These procedures are described below and a discussion is given of the data resulting from their use.

Experimental. CHEMICALS. A stock solution of selenium dioxide was prepared in the following manner. Eight grams of a technical grade of metallic selenium were dissolved in concentrated nitric acid, and the nitric acid was evaporated. The resulting tetrapositive selenium was twice distilled from a concentrated hydrobromic acid solution in order to separate the selenium from tellurium and other elements which are not volatile under these conditions (*12*). Sulfur dioxide was then passed through the distillate until there was no further precipitation. The resulting elemental selenium was filtered and again dissolved in concentrated nitric acid. The nitric acid was evaporated, and the resulting selenium dioxide was twice sublimed. This purified selenium dioxide was dissolved in a liter of boiled distilled water and standardized according to the method of Coleman and McCroskey (*1*).

Sodium thiosulfate solutions 0.1*VF* (volume formal, formula weights per liter, concentrations are used to avoid the uncertainty in the assignment of a normal concentration to thiosulfate used for Reaction 2) were standardized against potassium iodate.

Dilute standard solutions of selenious acid and thiosulfate were prepared by appropriate dilution of the above stock solutions.

All other chemicals used were reagent grade.

VOLUMETRIC TITRATION PROCEDURES. The two procedures described below were used. Procedure A is probably more accurate but requires a knowledge of the approximate position of the end point.

Procedure A. The desired volume of selenious acid solution was pipetted into an Erlenmeyer flask, 5 ml. of 6*VF* hydrochloric acid were added, and the volume was adjusted to approximately 30 ml. This solution was titrated with thiosulfate to within about 1 ml. of the end point. Then 5 ml. of starch indicator solution and 0.5 to 1 ml. of 1.0*VF* potassium iodide were added, and the titration was continued to a starch end point.

When this procedure is followed, only a slight amount, if any, elemental selenium is apparent at the end point.

Procedure B. After pipetting the desired volume of selenious acid into the flask and adding the hydrochloric acid and water, 5 ml. of starch indicator solution and 2 drops (approximately 0.05 ml.) of 1.0*VF* potassium iodide were added. The solution was then titrated with thiosulfate. When the blue color of the starch iodine complex disappeared, two drops more of 1.0*VF* potassium iodide were added, and, if the color reappeared, the titration was continued until a permanent end point was reached.

Discussion of Results. The experimental results obtained with Procedures A and B are shown in Table I. The quantities of selenium taken are calculated on the basis of the standardization of the selenious acid solution by the method of Coleman and McCroskey (*1*). The agreement is within the volumetric error for both procedures.

In outlining Procedure B, it was stated that before the end point was reached, the starch-iodine color disappeared. If the solution was then allowed to stand, the color would gradually reappear. More iodide was added in order to save time.

Although Procedures A and B yield comparable results, Procedure A is probably more accurate, as the results become low if Procedure B is modified by adding more iodide initially. When 1 meq. of iodide was added to 2.9 meq. of selenium, the error was −0.7%. When 2 meq. of iodide were added initially to the same quantity of selenium, the error increases to −1.1%. Thus, the amount of iodide added should be kept as small as possible.

Table I. Direct Volumetric Titration of Selenious Acid with Thiosulfate to the Starch End Point

Procedure	Selenium, Mg. Taken	Found	Error	Error, %
A	141.6	141.6	0.0	0.0
		141.6	0.0	0.0
	56.76	56.72	−0.04	−0.07
		56.67	−0.09	−0.16
	3.537	3.540	0.003	0.09
B	141.6	141.7	0.1	0.07
	56.76	56.72	−0.04	−0.07
	3.537	3.538	0.001	0.03

COULOMETRIC DETERMINATION OF MICROGRAM QUANTITIES OF SELENIUM

The coulometric titration of millinormal thiosulfate solutions in 0.1*VF* hydrochloric acid (*14*) suggested the possibility that microgram quantities of selenious acid might be determined by adding an excess of standard millinormal thiosulfate solution and titrating the excess with electrolytically generated iodine.

Two procedures have been studied. In one the thiosulfate is added to the selenious acid solution before addition of iodide whereupon selenopentathionate is formed according to Reaction 2. In the other the iodide is added before the thiosulfate, and selenium and iodine are formed according to Reaction 1; the thiosulfate then reduces the iodine.

Quantities of selenium ranging from 14 to 1400 γ have been determined by both of these methods and the results are shown below. The end points were determined amperometrically.

Experimental. Apparatus. The coulometric and amperometric apparatus has been described (*8, 13*). The generation rates used were 1.037×10^{-7} and 1.036×10^{-8} equivalent per second. A galvanometer the sensitivity of which was set to 0.5 μa. per division by appropriate shunting was used in measuring the indicator currents. A potential of 200 mv. was applied between the platinum indicator electrodes.

Coulometric Titration Procedures. Two procedures, C and D, were used. In both procedures 10 ml. of selenious acid solution and 5 ml. of 1.0*VF* hydrochloric acid were pipetted into a titration cell, followed by 20 ml. of distilled water. The order of addition of thiosulfate and iodide differed in the two procedures.

In Procedure C, 10 ml. of dilute standard thiosulfate solution of appropriate concentration were next pipetted into the titration cell followed by 5 ml. of 1.0*VF* potassium iodide; immediately thereafter the excess thiosulfate was titrated coulometrically with iodine. Selenopentathionate is formed in this procedure.

In Procedure D, 5 ml. of 1.0*VF* potassium iodide were next added to the titration cell, followed by the thiosulfate solution, and the titration was then made. Elemental selenium is formed in this procedure.

Blank thiosulfate titrations were run on solutions which were of similar composition, except that the selenious acid was replaced with 10 ml. of distilled water. With the exception of the blanks for the determination of 1400 γ quantities of selenium, these blank thiosulfate titrations agreed within 0.1% with those calculated from the volume and normality of the dilute thiosulfate solution added and from a blank run on the potassium iodide and hydrochloric acid solutions alone.

When blank titrations for 1400 γ quantities of selenium were made, a 1% excess of iodine was required. This error is attributed to decomposition of thiosulfate to sulfite prior to titration, since at the beginning of these blank titrations the thiosulfate is 0.002*VF* and similar errors have been found in previous experiments (*14*) with solutions having approximately the same thiosulfate and acid concentrations. When 1400 γ quantities of selenium were being determined, the excess of thiosulfate was only about one third of the total quantity added, and since the iodine generation time for a selenite titration was only about one third of that for a blank titration, the decomposition of thiosulfate, and, therefore, the error during such a selenite titration should be much less than that during a blank titration. Therefore, for such quantities of selenium, blanks were run on the potassium iodide and hydrochloric acid, and the equivalents of thiosulfate added were calculated on the basis of the dilution of the standard stock solution of sodium thiosulfate. That larger errors were not observed when these quantities of selenium were determined is possibly due to a cancellation of errors.

In all titrations and blanks the final volume was 50 ml., and the concentrations of hydrochloric acid and potassium iodide were each 0.1*VF*.

Discussion of Results. The results of determinations by the above procedures are shown in Tables II and III. With the exception of the 14 γ quantities, all errors are positive and are consistently larger when elemental selenium is formed (Procedure D) than when it is absent (Procedure C). The per cent error is roughly constant for both procedures. The observation of positive errors in titrations in which elemental selenium was formed was unexpected, since negative errors are observed in similar macro volumetric titrations (*2, 9*).

Table II. Selenopentathionate Formed in Coulometric Determination of Selenious Acid Using Procedure C

No. of Titrations	Selenium, γ Taken	Found	Error	Mean deviation	Error, %
		Oxygen Not Excluded			
5	1415	1418	3	0.6	0.2
5	283.0	283.8	0.8	0.1	0.3
5	160.1	160.1	0.0	0.1	0.0
5	141.3	141.4	0.1	0.1	0.1
3	14.11	14.07	0.04	0.04	−0.3
		Oxygen Excluded from All Solutions			
3	283.2	283.6	0.4	0.3	0.1

Table III. Elemental Selenium Formed in Coulometric Determination of Selenious Acid Using Procedure D

No. of Titrations	Selenium, γ Taken	Found	Error	Mean deviation	Error, %
		Oxygen Not Excluded			
3	1415	1423	8	0.9	0.6
4	283.0	285.1	2.1	0.1	0.7
5	160.1	160.5	0.4	0.1	0.3
5	141.3	142.0	0.7	0.1	0.5
3	14.11	14.07	−0.04	0.0	−0.3
		Oxygen Excluded from All Solutions			
5	283.2	283.6	0.4	0.2	0.1

The consistency of the positive errors suggests that there is an induced oxygen error. In order to check this possibility, titrations were made by Procedures C and D under oxygen-free conditions. All solutions were prepared oxygen-free by sweeping the water from which they were prepared with nitrogen. The titrations were then carried out under an atmosphere of carbon dioxide. The results of these determinations are shown at the bottoms of Tables II and III. The error resulting from both methods is decreased to about 0.1%; this indicates that the error is due to oxygen.

Procedure C, in which selenopentathionate is formed, is subject to a smaller oxygen error than is Procedure D; therefore its use is recommended.

Effect of pH. The coulometric determination of selenium was attempted at only one other pH value. When the solution was buffered at a pH of 3, there was no indication of any reaction between the thiosulfate and the selenious acid in the time required for a titration; the same number of reducing equivalents were found as were found in a titration of the thiosulfate alone.

ACKNOWLEDGMENT

Fred C. Anson has rendered valuable aid in carrying out certain of the coulometric titrations. Keith Rowley is indebted to the Dow Chemical Co. for a fellowship for the academic year 1952–1953 and to the Du Pont Co. for summer research assistance.

LITERATURE CITED

(1) Coleman, W. C., and McCroskey, C. R., Ind. Eng. Chem., Anal. Ed., 9, 431 (1937).

(2) Gooch, F. A., and Reynolds, W. G., *Z. anorg. Chem.*, **10**, 248 (1895).
(3) Klason, P., and Mellquist, H., *Z. angew. Chem.*, **25**, 514 (1912).
(4) Klein, A. K., *J. Assoc. Offic. Agr. Chemists*, **24**, 363 (1941).
(5) McCullough, J. D., Campbell, T. W., and Krilanovich, N. J., ANAL. CHEM., **18**, 638 (1946).
(6) McNulty, J. S., *Ibid.*, **19**, 809 (1947).
(7) McNulty, J. S., Center, E. J., and MacIntosh, R. M., *Ibid.*, **23**, 123 (1951).
(8) Meier, D. J., Myers, R. J., and Swift, E. H., *J. Am. Chem. Soc.*, **71**, 2340 (1949).
(9) Moser, L., and Prinz, W., *Z. anal. Chem.*, **57**, 277 (1918).
(10) Muthman, W., and Schaefer, W., *Ber.*, **26**, 1008 (1893).
(11) Norris, J. F., and Fay, H., *Am. Chem. J.*, **18**, 705 (1896).
(12) Noyes, A. A., and Bray, W. C., "Qualitative Analysis for the Rare Elements," p. 37, Macmillan, New York, 1927.
(13) Ramsey, W. J., Farrington, P. S., and Swift, E. H., ANAL. CHEM., **22**, 332 (1950).
(14) Rowley, K., and Swift, E. H., *Ibid.*, **26**, 373 (1954).
(15) van der Meulen, J. H., *Chem. Weekblad*, **31**, 333 (1934).
(16) Wernimont, G., and Hopkinson, F. J., IND. ENG. CHEM., ANAL. ED., **12**, 308 (1940).

RECEIVED for review September 2, 1954. Accepted October 23, 1954. Contribution No. 1935 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena 4, Calif.

Chromatographic Separation and Determination of Porphyrin Methyl Esters

D. A. RAPPOPORT, C. R. CALVERT, R. K. LOEFFLER, and J. H. GAST

Departments of Radiology and Biochemistry, Baylor University College of Medicine, Houston 25, Tex.

By means of horizontal paper chromatography with filter paper placed between glass plates, the methyl esters of uroporphyrin I, coproporphyrins I and III, and protoporphyrin IX were separated within 1 hour. Elution of individual bands from the paper chromatogram followed by fluorometric analyses permitted quantitative estimations of each porphyrin ester.

DURING an investigation of various methods for the separation and quantitative determination of porphyrin isomers, difficulties were experienced in separating for analyses sufficient amounts of various porphyrins from biological fluids. In this laboratory, repeated clear-cut separations of porphyrin methyl esters from biological fluids with the paper chromatographic methods of Chu, Green, and Chu (*2*) and the modifications of this method by Bogorad and Granick (*1*) was found to be difficult because of interference by impurities. The resultant bands of the porphyrin esters, from either ascending or descending chromatograms, were somewhat irregular and overlapping and at best insufficiently separated for quantitative recoveries.

Using horizontal paper chromatography with a modified solvent system, a rapid method for the complete separation of methyl esters of uroporphyrin I, coproporphyrin I, coproporphyrin III, and protoporphyrin IX has been developed which permits quantitative estimation of each of these porphyrin esters.

EQUIPMENT AND MATERIALS

Two glass plates, 13 inches square, cut from 0.25-inch commercial plate glass. Any other suitable size may be used. One glass square is marked at the exact center with a wax pencil, and a hole 0.25 inch in diameter is drilled through. This is done by forming a ridge of putty around the center mark so that water can stand over this area. Using a short length of 0.25-inch brass tubing in an electric drill press and adding abrasive powder intermittently, a hole is drilled halfway through on one side of the plate, then completed by drilling from the opposite side.

Two barrels from hypodermic syringes, 10 or 20 ml., each of which is fitted with a piece of thin-walled (5-mm. outside diameter) gum rubber tubing over the entire length of the syringe tip. Each syringe is packed with Whatman cellulose powder to a depth of about 2 cm. Before use, each packed syringe is rinsed with the particular solvent to be used for chromatography, and the rate of solvent flow is adjusted to approximately 1 drop per 2 seconds by tamping the cellulose packing with a glass rod.

Six lead bricks, weighing about 25 pounds each (Nuclear Instrument and Chemical Corp., Chicago, Ill.).

Filter paper sheets, Whatman No. 3MM, cut in squares (13 × 13 inches) the same size as the glass plates. At the center of each paper the location for the sample is made by drawing a circle 1 inch in diameter. This circle is divided into arcs by two to six radial lines allowing as many as six samples to be chromatographed simultaneously.

Developing Solvents. SOLVENT A. Three volumes of petroleum ether (boiling point 30° to 60° C.) and 1 volume of chloroform.

SOLVENT B. Twenty volumes of heptane, 1 volume of ethylene dichloride, and 1.5 volumes of *tert*-butyl alcohol.

AUXILIARY SOLVENT. Equal volumes of petroleum ether and chloroform.

Methyl esters of coproporphyrins I and III and of uroporphyrin were furnished by Samuel Schwartz. Paper chromatographic analysis indicated that the esters were single components with only traces of impurities. The methyl ester of protoporphyrin IX was prepared from crude protoporphyrin (H. M. Chemical Co., Ltd., Santa Monica, Calif.) and purified by means of paper chromatography.

Ultraviolet lamp, 360 mμ, Hanovia Type 1C103 (Hanovia Chemical and Mfg. Co., Newark, N. J.).

Fluorometer, Lumetron Model 402-EF, used with a primary filter of Corning glass 5543 (365 mμ) and secondary filter of Cor-

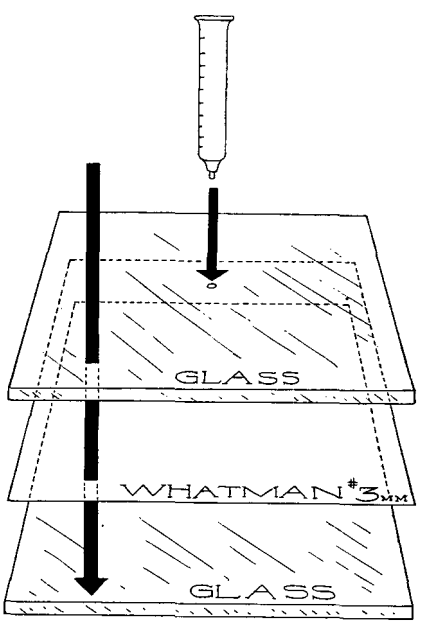


Figure 1. Schematic representation apparatus used in horizontal paper chromatography

ning glass No. 2418 (600 mμ) (Photovolt Corp., New York, N. Y.).

Calibrated micropipets, Levy type (Microchemical Specialties Co., Berkeley, Calif.).

PROCEDURE

Porphyrins are isolated from biological fluids by an ether–ethyl acetate extraction (*4*), by calcium phosphate precipitation (*5*), or by lead salt precipitation (*3*). The mixture of porphyrins is esterified by dissolving the crude extract in 3 to 4 volumes of methanol saturated with hydrogen chloride gas, and the solution is allowed to stand overnight at room temperature in the dark (*1, 5*). Five volumes of water are added to the methanol–hydrochloric acid and the porphyrins are extracted from the diluted solution into chloroform. The chloroform phase is separated, washed with 10% sodium acetate solution, then with water, decanted into a dry beaker, and concentrated in a vacuum desiccator to a volume of 0.5 to 2.0 ml.

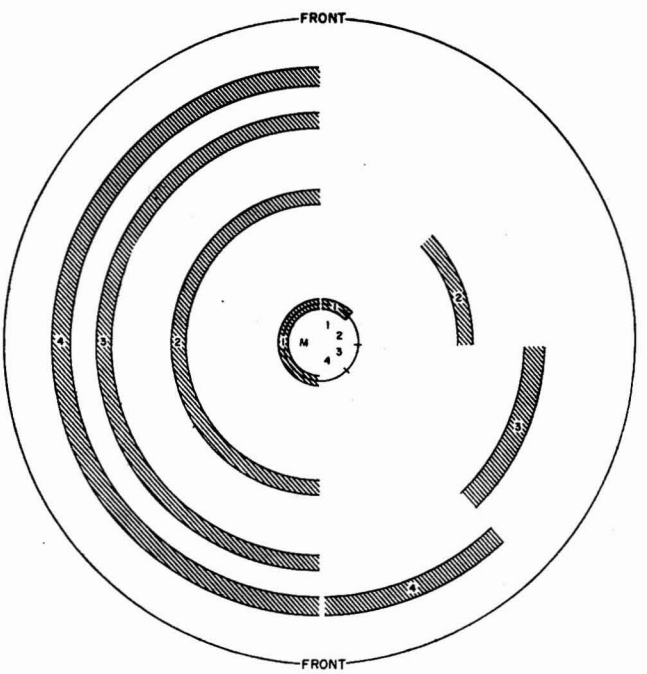


Figure 2. Chromatographic separation of methyl esters of uroporphyrin I (1), coproporphyrin I (2), coproporphyrin III (3), and protoporphyrin IX (4)

Position of individual porphyrin esters is shown to the right; on left is separation from mixture (*M*)

Depending on the concentration of porphyrin esters in the final chloroform solution, 50 to 200 μl. of this solution is transferred by means of a calibrated micropipet to the marked position on the filter paper. The sample is carefully placed on the sample line to form a narrow curved band. As many as six samples can be transferred to one sheet of paper in this manner. When dry, the paper is placed between two glass plates with the center of the paper in line with the opening in the upper plate, and the syringe is then securely fitted into the opening. Figure 1 illustrates the position of the relevant parts. Six lead bricks are placed on the top of the glass plates close to the syringe so that there is a uniform pressure on the paper.

About 2 ml. of solvent A is transferred to the syringe and the chromatogram is allowed to develop until the solvent front is approximately half way across the paper. The filter paper is then removed and allowed to dry in air. The paper is again placed between the glass plates, a new packed syringe is fitted into the opening, and the plates are weighted with the lead bricks. About 8 ml. of solvent B are transferred to the syringe and the chromatogram is allowed to develop until the solvent reaches the edge of the paper. The filter paper is then removed, air dried, and examined under the ultraviolet lamp so that the position of each band can be marked. Figure 2 illustrates the relative position of the porphyrin methyl ester after chromatography by the described procedure. The R_f values are as follows:

Uroporphyrin I octamethyl ester	0
Coproporphyrin I tetramethyl ester	0.53
Coproporphyrin III tetramethyl ester	0.62
Protoporphyrin IX dimethyl ester	0.83

Using solvent B only, the methyl esters of protoporphyrin and coproporphyrins I and III are separated. Figure 3 illustrates a chromatogram developed with solvent B. This solvent does not separate the methyl esters of coproporphyrin I and uroporphyrin I, since both have an R_f of zero.

If porphyrin extracts contain large amounts of impurities, such as are normally found in extracts from urine, it is necessary to use the auxiliary solvent as the first developing solvent to separate the porphyrin esters from the impurities. If 2 ml. of auxiliary solvent are transferred to the syringe, the porphyrin esters will move about 1 inch from the origin along with the solvent front. After the paper is dry, use of solvents A and B, respectively, as described previously, will result in a complete separation of the porphyrin methyl esters into distinct bands, whereas the impurities will remain at the origin. Figure 4 illustrates such a chromatogram of porphyrin esters from a urine to which uroporphyrin I, coproporphyrins I and III, and protoporphyrin IX were added before extraction, esterification, and chromatography. The R_f values are as follows:

Uroporphyrin I octamethyl ester	0.13
Coproporphyrin I tetramethyl ester	0.55
Coproporphyrin III tetramethyl ester	0.70
Protoporphyrin IX dimethyl ester	0.91

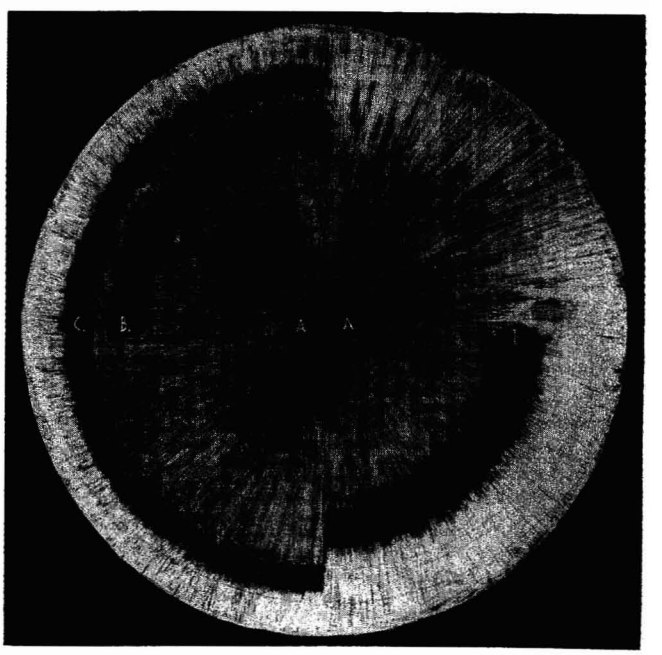


Figure 3. Drawing of actual chromatogram, made under ultraviolet illumination of methyl esters of coproporphyrin I (*A*), coproporphyrin III (*B*), and protoporphyrin IX (*C*) developed with solvent B

For quantitative determination of each porphyrin ester after chromatography, each band is cut from the paper, the paper shredded, and placed in test tubes (180 × 15 mm). After addition of 15 ml. of 1.5*N* hydrochloric acid, the test tubes are placed in the dark for 30 minutes or longer with occasional shaking. Subsequently, the acid solution is decanted into clean test tubes for fluorometric reading. Standard curves were established with methyl esters of protoporphyrin IX, coproporphyrin III, and uroporphyrin I, as shown in Figure 5. Two filters were used, a monochromatic primary filter with a maximum transmittance at 365 mμ, and a secondary filter transmitting above 600 mμ.

Table I. Recovery of Porphyrin Methyl Esters from Known Mixtures

	Protoporphyrin IX			Coproporphyrin III			Uroporphyrin I		
Sample	Present, γ	Recovered γ	Recovered %	Present, γ	Recovered γ	Recovered %	Present, γ	Recovered γ	Recovered %
1	30	26	87	30	27	90	28	27	97
2	30	25	84	30	27	90	28	27	97
3	30	26	87	30	27	90	28	27	97
4				30	28	93	28	27	97
Average			86			91			97

RESULTS

Tests of the above quantitative chromatographic procedure with known mixtures of pure methyl esters of protoporphyrin IX, coproporphyrin III, and uroporphyrin I gave recoveries of 86, 91, and 97%, respectively, as shown in Table I.

It is apparent from the above data that with increased R_f values, lesser amounts of the porphyrin methyl esters are recovered. This is probably due to tailing along the paper during chromatography, which becomes more significant with components that travel the greatest distance from the origin.

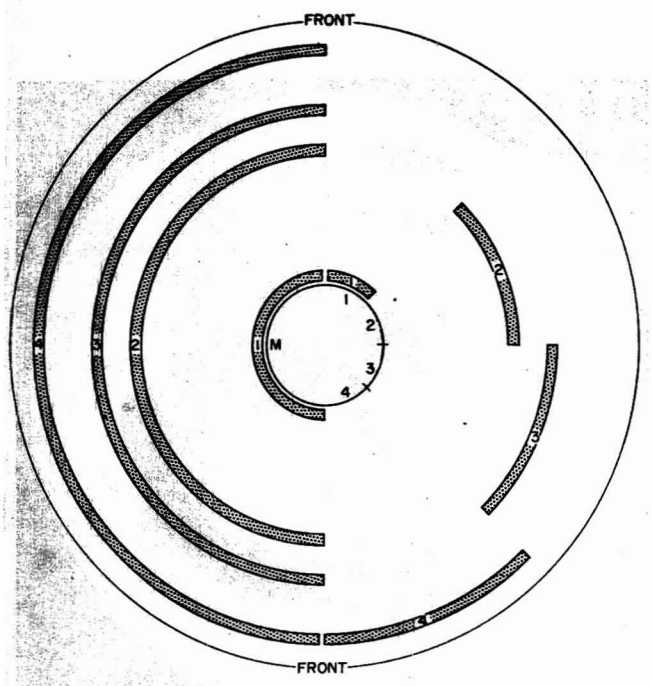


Figure 4. Diagram of chromatogram, made under ultraviolet illumination, of porphyrin methyl esters from urine

Numbers designate same components as in Figure 2

Recovery experiments were made with coproporphyrin added to different urine samples. Table II shows the results of these experiments. The average recovery of coproporphyrin in these experiments was 96% with a range of ± 3%.

In general this procedure for separation and determination of porphyrins showed an accuracy within ± 3% and a sensitivity of approximately 0.1 γ.

The entire process was carried out under ordinary diffuse room lighting. Care was taken to protect the materials from direct sunlight, but no special effort was made to protect them from room light during the time required for chromatography and drying. No losses were observed under diffuse light (3 to 4 hours) although exposure to sunlight caused appreciable loss.

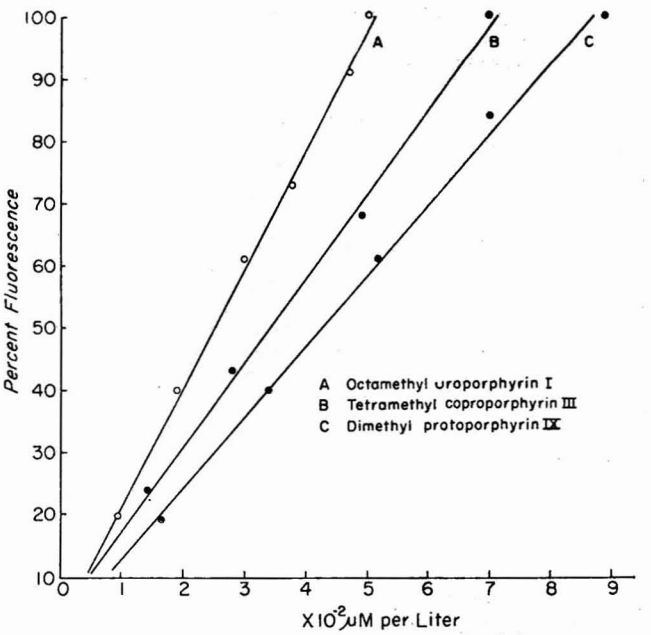


Figure 5. Fluorescence-concentration relation of porphyrin methyl esters

After development of the chromatograms and drying, they were placed in a drawer and covered with filter paper, as recovery experiments indicated a loss of pigment or fading within 24 hours if left in room light. Those placed in desk drawers, however, gave the same recovery as long as 3 days after completion of the chromatogram.

Table II. Recovery of Coproporphyrin III from Urine

Sample	Original Concentration, γ	Added, γ	Total Concentration, γ	Recovered γ	Recovered %
1	157	30	187	180	96
2	189	30	219	216	99
3	2.5	28	31.5	29	95
4	13	28	41	38	93
			Average		96

ACKNOWLEDGMENT

The authors wish to thank Samuel Schwartz, Medical School, University of Minnesota, for his generosity in supplying pure samples of the methyl esters of uroporphyrin I, and coproporphyrins I and III. Thanks are also due to John Oró for his suggestions on horizontal chromatography.

The major portion of this investigation was supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, Contract No. Da-49-007-MD-428.

LITERATURE CITED

(1) Bogorad, Lawrence, and Granick, S., *J. Biol. Chem.*, 202, 793 (1953).
(2) Chu, T. C., Green, A. A., and Chu, E. J., *Ibid.*, 190, 643 (1951).
(3) Corwin, L. M., and Orten, J. M., ANAL. CHEM., 26, 608 (1954).
(4) Dobriner, K., *J. Biol. Chem.*, 113, 1 (1936).
(5) Nicholas, R. E. H., and Rimington, C., *Scand. J. Clin. & Lab. Invest.*, 1, 12 (1949).

RECEIVED for review November 30, 1954. Accepted January 17, 1955.

Determination of Microgram and Submicrogram Quantities of Uranium by Neutron Activation Analysis

H. A. MAHLMAN and G. W. LEDDICOTTE
Oak Ridge National Laboratory, Oak Ridge, Tenn.

Microgram and submicrogram quantities of uranium have been determined in synthetic samples, ores, and soils by neutron radioactivation analysis. The principles of the activation analysis method used in this determination and the processing of irradiated samples are discussed. This method of analysis is a sensitive and specific method for determining uranium in concentrations as small as 0.1 γ per gram with a probable relative standard error of 10%. Concentrations of uranium in quantities as small as 0.0001 γ per gram can be determined by neutron activation analysis.

THE rapid development of nuclear science in recent years has brought into existence a new method of analysis for the assay of trace elements contained in many different materials. Known as radioactivation analysis, the method is based on the measurement of nuclear radiations, either α, β, or γ, from radioactive isotopes that are induced artificially in the stable isotope(s) of an element by interactions with the nuclear particles (such as neutrons and deuterons) produced either in a chain-reacting pile or in a charged particle accelerator.

Radioactivation analysis is extremely sensitive for most elements. It is also specific, because each induced radionuclide has its own particular decay constant and type(s) of radiation, neither of which is exactly duplicated in any other radionuclide. Contamination, as experienced in most conventional methods of analysis, is negligible, because the contaminants must be present before nuclear irradiation in order to undergo nuclear reaction.

The fundamentals of radioactivation analysis have been discussed by Boyd (*2*) and by Taylor and Havens (*8*). More recently, Leddicotte and Reynolds (*3*, *4*) have reported on the use of the Oak Ridge National Laboratory (ORNL) graphite reactor to determine submicrogram and microgram amounts of many elements by neutron activation analysis. (An analytical service by radioactivation analysis is now a part of the Oak Ridge National Laboratory program). At least 70 elements can be determined in a variety of materials with sensitivities of detection ranging from 0.00001 to 1 γ by the neutron activation analysis method. Neutron radioactivation analysis at ORNL has been used to determine microgram and submicrogram concentrations of uranium in synthetic samples, ores, and soils.

DETERMINATION OF URANIUM BY NEUTRON RADIOACTIVATION ANALYSIS

Nuclear Data. The reactions of the uranium isotopes, uranium-234, uranium-235, or uranium-238 with thermal neutrons—i.e., neutrons having an energy of 0.0252 ev.—can be used in this method of analysis. The percentage abundances of these isotopes are 0.006, 0.72, and 99.274%, respectively. The reactions of these isotopes with thermal neutrons are summarized as follows:

$$^{234}_{92}\mathrm{U}\ (n,\gamma)\ ^{235}_{92}\mathrm{U} \qquad (1)$$

$$^{234}_{92}\mathrm{U}\ (n,\gamma)\ \text{fission products} \qquad (2)$$

$$^{238}_{92}\mathrm{U}\ (n,\gamma)\ ^{239}_{92}\mathrm{U} \xrightarrow[23.5\mathrm{m}]{\beta^-}\ ^{239}_{93}\mathrm{Np} \xrightarrow[2.33\mathrm{d}]{\beta^-}\ ^{239}_{94}\mathrm{Pu} \xrightarrow[23{,}360\mathrm{y}]{\alpha}\ ^{235}_{92}\mathrm{U} \qquad (3)$$

In Reaction 1, the amount of uranium-235 activity produced from uranium-234 is negligible when short-time irradiations are used because the half-life of uranium-235 is of the order of 9×10^8 years, and the percentage abundance of the uranium-234 isotope is very low.

Smales (*7*) and Seyfang and Smales (*6*) have used Reaction 2 to determine total uranium in minerals and uranium-235 in uranium mixtures. The uranium content of the samples can be determined by measuring the amount of barium-140 radioactivity produced in a predetermined time of irradiation relative to a comparative standard. Barium-140 ($t_{1/2}$ = 12.8 days) emits both β and γ radiation. A radiochemical separation of the barium-140 is usually made from a solution of the irradiated material.

Reaction 3 was used in the work reported herein. Either the uranium-239 activity, or the neptunium-239 daughter activity, or the plutonium-239 daughter activity produced in this reaction is proportional to the amount of uranium present in the sample. The short half-life of uranium-239 (23.5 minutes) necessitates a rapid radiochemical separation. However, rapidity is not always conducive to an efficient removal of the contaminant radioactivities produced from other elements that may be present in the sample. On the other hand, it would take considerable time to obtain sufficient plutonium-239 α activity from the decay of the plutonium-239 for good sensitivity. Thus, neptunium-239 ($t_{1/2}$ = 2.33 days) was considered as the best radionuclide to use in a radiochemical separation procedure.

Determination of Neptunium-239 Radioactivity. As neptunium-239 decays, it emits both β and γ radiations. The radionuclide has a spectrum of 7 γ (*9*), the energies of which are in the range of 0.057 to 0.50 mev. Radiations of such energy can be conveniently detected by means of a γ scintillation counter having a sodium iodide crystal (thallium activated) (*1*). The β radiations can be detected by means of a Geiger-Müller counter.

Comparative Method of Analysis. The amount of neptunium-239 measured by gamma counting is directly proportional to the initial amount of uranium-238 in the sample. If the sample and a comparative standard [a known weight of uranium oxide (U_3O_8)] are irradiated simultaneously, processed chemically, and counted under similar conditions, then the amount of uranium in a sample can be calculated as follows:

$$\text{Weight of uranium in sample} = \frac{\text{Corrected count of Np-239}}{\text{corrected count of Np-239 per gram of uranium in comparator}}$$

where the corrected counts include corrections for counter background and the decay of neptunium-239 radioactivity in both sample and comparative standard.

Because the amount of neptunium-239 formed is dependent upon the concentration of uranium-238 present in the irradiated sample, it can be readily seen that any alteration in the natural ratio of the uranium isotopes will cause bias results. The bias is limited on uranium-235 depleted uranium but may be serious where the opposite situation is present.

The necessity of monitoring the flux of neutrons, which is usually a difficult quantity to measure or control exactly, is eliminated by the use of the comparative sample.

Neutron Source. The ORNL graphite reactor was used as the neutron source for all the work described herein. The neutron flux of this reactor is about 10^{12} neutrons per sq. cm. per second.

Self-Shadowing. It has been recognized that a neutron flux depression occurs at the center of the sample undergoing irradiation; this is called self-shadowing. The self-shadowing effects are negligible if solid samples are irradiated in 4-mm. inside diameter quartz tubing. Liquids are irradiated in quartz ampoules and contain low solute concentrations, thus attenuation of the neutron flux is similar to that of water. The presence in the sample of other elements which may have high neutron-absorption cross sections will result in low results. For example, gadolinium would cause serious neutron flux attenuation in the sample and would necessitate a preirradiation separation of the uranium by some convenient quantitative method.

RADIOACTIVATION ANALYSIS OF SAMPLES THAT CONTAIN URANIUM

Nuclear Irradiation of Sample. Weighed portions of the samples and the comparative standard are put into small quartz tubes. The tubes are closed with cork stoppers that are wrapped in aluminum. They are then irradiated in the reactor. After irradiation, the samples are allowed to decay about 4 hours and are then chemically processed as described below. The synthetic samples used in this laboratory had been processed by a filter paper partition chromatography technique. After the separation, the paper was conveniently irradiated in short pieces of quartz tubing whose openings were plugged by means of cork stoppers.

Chemical Separation of Neptunium-239. In most neutron activation analyses, a chemical separation is made to isolate the radioactivity of the element from all other radioactive species in the sample. Usually an "isotopic carrier"—a known amount of the natural inactive element—is added to the solutions of both the irradiated specimen and the comparison samples. The solutions are then processed chemically to isolate the carrier and desired radioelement from other elements and contaminant radioactivities. Small amounts of other elements are added as holdback or scavenging carriers to assist in the decontamination process.

Although neptunium-239 has a convenient half life, it does not have a stable isotope that can be used as an isotopic carrier. However, Seaborg (*5*) has shown that trace quantities of neptunium-239 can be quantitatively carried on a nonisotopic carrier, such as cerium. The method of analysis reported below uses lanthanum as a nonisotopic carrier for the neptunium-239 radioactivity.

Chemical Separation Procedure. PREPARATION. The irradiated ore and soil specimens are dissolved by digestion in a mixture of concentrated nitric, hydrofluoric, perchloric, and sulfuric acids. (Additional hydrofluoric acid can be added if a residue of silica remains in the bottom of the crucible.) After dissolution, the sample is concentrated to heavy sulfuric acid fumes, cooled, and transferred to a 15-ml. centrifuge tube. If a residue (sulfate salts) remains after the transfer, the solution is centrifuged for 5 minutes, the supernatant transferred to another tube, and the residue washed with 1 ml. of 1*M* nitric acid. The wash is added to the supernatant and the residue discarded. (Centrifugation is always for the stipulated time and at full speed.) The sample is then further processed by the procedure reported herein.

The irradiated synthetic samples (paper chromatograms) are processed by carefully igniting the paper contained in a porcelain crucible in a muffle furnace. The residue is dissolved in about 0.5 ml. of concentrated nitric acid. After dissolution, the sample is transferred to a 15-ml. centrifuge tube and the processing continued with the procedure reported herein.

PROCEDURE. Three (3.0) milligrams of lanthanum and 0.250 ml. of 5*M* hydroxylamine hydrochloride solution are added to the supernatant solution and the mixture digested for 5 minutes with occasional stirring. The solution is cautiously neutralized with concentrated ammonium hydroxide to precipitate lanthanum hydroxide, after which the mixture is centrifuged and the supernatant liquid discarded.

The precipitate of lanthanum hydroxide is dissolved in 2 ml. of 2*M* hydrochloric acid, and 1.0 mg. of strontium (added as a solution of strontium nitrate to serve as a holdback or scavenging carrier) and 0.250 ml. of 5*M* hydroxylamine hydrochloride solution are added to the solution. The solution is again digested for 5 minutes with intermittent stirring, and 0.200 ml. of concentrated hydrofluoric acid is added dropwise to the solution to precipitate lanthanum fluoride. After centrifugation, the supernatant liquid is discarded and the precipitate washed with 0.5 ml. of 1*M* hydrofluoric acid–1*M* nitric acid solution.

After washing, the lanthanum fluoride precipitate is dissolved in 0.5 ml. of saturated boric acid solution and 1.0 ml. of 6*M* nitric acid. One (1.0) milliliter each of 10% potassium permanganate solution and water are added to this solution, and the resulting mixture is agitated well and digested for 5 minutes. Lanthanum fluoride is again precipitated with 0.250 ml. of concentrated hydrofluoric acid; the solution is centrifuged and the supernatant liquid transferred to another centrifuge tube. The precipitate is washed with 0.5 ml. of 1*M* hydrofluoric acid–1*M* nitric acid solution and the wash combined with the supernatant liquid. The precipitate is discarded.

Three milligrams of lanthanum are added to the supernatant liquid, and the solution is digested for 5 minutes and centrifuged. An additional 3.0 mg. of lanthanum are added to the supernatant liquid and the solution agitated and digested for 5 minutes without disturbing the first precipitate on the bottom of the tube; then the solution is centrifuged and the supernatant liquid transferred to another centrifuge tube. The precipitate is washed with 0.5 ml. of 1*M* hydrofluoric acid–1*M* nitric acid solution, centrifuged, and the wash combined with the supernatant liquid. The precipitate is discarded.

One milligram of zirconium (added as a solution of zirconium nitrate to serve as a holdback or scavenging carrier) and 0.250 ml. of 5*M* hydroxylamine hydrochloride are added to the solution and the mixture agitated and digested 5 minutes. Three (3.0) milligrams of lanthanum and 2 ml. of 2*M* hydrofluoric acid are added to the solution, and the solution is digested for 20 minutes and then centrifuged. The supernatant liquid is discarded. The precipitate is washed with 0.5 ml. of 1*M* hydrofluoric acid–1*M* nitric acid solution, and the resulting mixture is centrifuged. The wash solution is discarded after the centrifugation.

The precipitate is slurried in a small amount of 1*M* nitric acid (about 0.5 ml.) and transferred to a small borosilicate glass culture tube by means of a transfer pipet. The centrifuge cone is rinsed with three 0.5-ml. portions of 1*M* nitric acid and the rinses transferred to the culture tube. The tube is stoppered with a cork stopper and the γ radioactivity measured by a well-type gamma scintillation counter.

The standard sample of uranium oxide (U_3O_8) is dissolved in nitric acid and an aliquot of the solution processed under the same conditions as the specimen samples. As discussed previously, the uranium content of the sample in question is determined by equating the ratio of the corrected neptunium-239 radioactivity count in the unknown and the corrected neptunium-239 radioactivity count in the standard sample.

Interferences in the Chemical Separation of Neptunium-239. The interferences that can be attributed to the presence of radioactivities associated with thorium and the fission products are negligible if two or more lanthanum fluoride precipitations are used to decontaminate the neptunium-239. A chemical interference was noted while precipitating lanthanum fluoride from the diluted dissolving solution. It was found that if the sulfuric acid concentration was less than 1.25*M* or if an ammonium hydroxide precipitation was included, this difficulty could be circumvented completely. The presence of phosphoric acid did not interfere.

Identification and Measurements of Separated Neptunium-239. The slurry of the lanthanum + neptunium-239 fluoride precipitate is placed in a small borosilicate glass culture tube and its radioactivity measured by means of a γ scintillation counter (*1*). In the experimental work, an identification of the neptunium-239 was made by aluminum absorption studies of the β radiations and by a decay, or half life, study of the radioactivity emitted by neptunium-239 as it decays.

Sensitivity of Detection. Such factors as the number of neutron particles bombarding the material, the weight of material irradiated, the length of irradiation, "self-shadowing," self-absorption, time for chemical separation, the activation cross section, and the efficiency of measuring the separated radioactivity reflect upon the sensitivity attainable by radioactivation analysis. Although this work primarily reports on the determination of concentrations of uranium of about 0.30 γ per gram or more, uranium can be determined in concentrations as small as 0.003 γ per gram. Most of the radioactivity measurements obtained in the analyses reported here were based on gamma counting rates of at least 100,000 γ-ray counts per minute. Thus, if one considers a counting rate of 1000 counts per minute including background as

Table I. Determination of Uranium in Synthetics by Neutron Activation Analysis

Determination	Uranium Taken, γ	Uranium Found, γ
1	0.34	0.33
2	0.34	0.31
3	0.34	0.33
4	0.34	0.32
5	0.34	0.36
6	0.34	0.34
7	0.34	0.35
8	0.34	0.35
9	0.34	0.30
10	0.34	0.30
		Mean 0.33
		Std. Dev. 2.70%

Table II. Determination of Uranium in Phosphate Ores

Determination	Sample 1, γ/Gram	Sample 2, γ/Gram	Sample 3, γ/Gram
1	171	288	172
2	161	261	164
3	171	262	158
4	161	275	149
5	158	249	148
6	168	256	180
7	158	271	166
8	163	240	164
9	160		
10	174		
11	166		
12	169		
Mean	165	263	163
Std. dev.	5.5	15.3	10.8
% Std. dev.	3.3	5.8	6.6
Reported fluorometric results	160	250	160

being the accuracy limit of the gamma counter, it is possible to determine at least 0.003 γ per gram. Increased sample size and longer periods of irradiation in the reactor would greatly enhance this sensitivity. Considering all of the factors given above, it has been calculated that at least 0.0001 γ per gram of uranium can be detected.

RESULTS

Reproducibility. The precision of analysis for uranium is within ±10%. The results reported in Tables I through III show the relative standard deviation for each set of determinations.

Determination of Uranium in Synthetics. This investigation of the method of radioactivation analysis for uranium was first applied to the determination of uranium in a series of synthetic samples. The handling of the samples before irradiation has been described above. The samples were irradiated for about 62 hours and processed by the procedure given in this report. Typical data obtained on this type of material are shown in Table I.

Determination of Uranium in Phosphate Ores. A series of phosphate ore samples was analyzed by irradiating portions (approximate weight = 0.025 gram) of the material for 3.0 hours in the ORNL graphite reactor. After the irradiation, the samples were dissolved and processed by the procedure given in this report. The results obtained are shown in Table II.

Determination of Uranium in Soils. Several soil samples were analyzed by irradiating duplicate portions of the materials for 62 hours in the ORNL graphite reactor. After irradiation, the samples were processed by the procedure described in this report. The results are reported in Table III.

Table III. Determination of Uranium in Soils by Neutron Activation Analysis

Sample	Uranium Concentration, γ/Gram
1	69.7, 68.2
2	48.3, 51.1
3	84.0, 80.0

ACKNOWLEDGMENT

The authors wish to acknowledge the work of T. C. Rains, J. H. Oliver, J. J. Manning, L. M. Frakes, N. B. Tuck, N. F. Sharp, and W. L. Bruce in analyzing the irradiated materials.

LITERATURE CITED

(1) Borkowski, C. J., ANAL. CHEM., **21**, 348 (1949).
(2) Boyd, G. E., *Ibid.*, **21**, 335–47 (1949).
(3) Leddicotte, G. W., and Reynolds, S. A., *Nucleonics*, **8**, No. 3, 62–5 (1951).
(4) Leddicotte, G. W., and Reynolds, S. A., *ORNL Rept.*, **1443** (Jan. 2, 1953); **AECD-3489** (declassified Jan. 27, 1953).
(5) Seaborg, G. T., and coworkers, *Metallurgical Project Rept.*, **CN-2689**, 41 (Feb. 15, 1945) (classified).
(6) Seyfang, A. P., and Smales, A. A., *AERE Rept.*, **C/R 980** (Oct. 3, 1952) (unclassified).
(7) Smales, A. A., *Ibid.*, **C/R 930** (May 13, 1952) (unclassified).
(8) Taylor, T. I., and Havens, W. W., Jr., *Nucleonics*, **6**, No. 4 54–66 (1950).
(9) Way, K., Fano, L., Scott, M., and Thew, K., Natl. Bur. Standards U. S., *Circ.* **499** (1950).

RECEIVED for review November 19, 1954. Accepted January 17, 1955.

Analysis of Automobile Exhaust Gases by Mass Spectrometry

J. K. WALKER and C. L. O'HARA, *Consolidated Engineering Corp., Pasadena 15, Calif.*

Mass spectrometer techniques for the analysis of automobile exhaust gases, and a sampling procedure for laboratory gas analysis are presented. These data are supplemented by results from continuous monitoring of exhaust gas composition at various engine speeds. The hydrocarbon content of automobile exhaust varies with engine speed, approaching steady state at high speed. The oxides of nitrogen produced, as indicated by continuous analysis, show an increase with engine speed.

ANALYSIS of exhaust gases from automobiles has long presented a problem in the study of air pollution. The mass spectrometer offers exceptional promise as a satisfactory means for complete analysis and monitoring of all combustion products from internal combustion engines. Mass spectrometric techniques for the determination of exhaust gases have been reported previously (*1, 5, 9*). The lubricating oil products of exhaust gases can be determined similarly (*8*), but are not considered as major contributors to air pollution by virtue of their lower vapor pressure.

While reporting results of recent mass spectrometric analyses of engine exhaust, the problems encountered in analysis and the sampling techniques available should be reviewed. The greatest single problem met in automobile exhaust gas analysis is that of obtaining representative sampling. Although 85% of the combustion products are gaseous at atmospheric pressure and ambient temperature, the other 15% are liquids condensed or adsorbed below the operating temperatures. Previous reports have indicated the condensable and soluble gaseous components other than water to be most significant in the problems of air pollution (*4, 10*).

Because the theoretical water content of exhaust gases (*7*) can be computed on the basis of the combustion products, the gasoline and soluble gas content of any one sample should be representative if the measured condensable water content is near theoretical. Providing complete vaporization of all condensable gases can be maintained, the limitations of the mass spectrometer and the problem of representative sampling can be somewhat overcome by continuous sampling directly from an operating engine into a mass spectrometer (*11*) (Figure 1).

SAMPLE ANALYSES

In general, the products of automobile exhaust may be separated into three classes: combustion products, hydrocarbons, and atmospheric gases. The combustion gases may be further classified as partial and complete oxidation products. It is also desirable to differentiate the cracked hydrocarbons from the gasoline. Table I lists three representative analyses taken from a 1954 standard-made automobile, with the engine idling. The water content varies from 8.5 to 24.2 mole % without appreciable effect upon the hydrocarbon content. Previously reported analyses (*5*) are low in gasoline content, when the water content of the vapor is below 5%.

From the analyses shown in Table I the water content of the combustion products can be computed to be 14.9%, assuming an average fuel composition of C_8H_{17} (*7*). Twelve actual samples showed an average water content of 16.7 mole %. Therefore, the water and fuel products of the exhaust gases were sampled representatively and fully vaporized into the mass spectrometer.

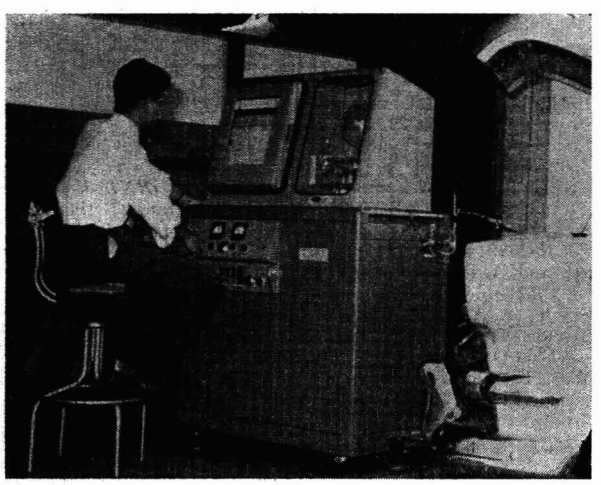


Figure 1. Mass spectrometer recording spectra of sample directly from operating engine

Table I. Composition of Automobile Exhaust Gases

(1000 r.p.m. idle)

	Mole %		
Combustion Products			
Partial			
Hydrogen	2.17	2.36	2.39
Carbon monoxide	4.76	4.90	5.04
Complete			
Carbon dioxide	11.6	11.4	11.0
Water[a]	24.2[a]	14.4[a]	8.53[a]
Alcohols[b]	0.02	0.01	0.02
Sulfur dioxide	0.06	0.06	0.05
Hydrogen sulfide	0.01	0.01	0.01
Hydrocarbons			
$<C_3$ cracked hydrocarbons	0.22	0.25	0.24
$>C_4$ gasoline	0.22	0.23	0.28
Atmospheric Gases			
Nitrogen	80.2	80.1	80.3
Argon	0.74	0.68	0.67
Air[a]	1.53[a]	2.23[a]	1.41[a]

[a] Analyses computed air- and water-free.
[b] Alcohols average of C_1 to C_3.

In addition to determining total hydrocarbon content of exhaust gases, it is important to know the compound types present—e.g., paraffins (C_nN_{2n+2}), olefins (C_nH_{2n}), cyclo-olefins, diolefins and acetylenes, and aromatics (benzene homologs). Assuming that all products containing three or more carbon atoms displayed in the mass spectrum of the mixture originate from unburned gasoline products, a modified gasoline-type analysis (*1*) can be performed. Similarly, this assumption can be made for all products of five or more carbon atoms. The difference between the C_3^+ and C_5^+ hydrocarbons should be indicative of the cracked products in this molecular weight range.

Table II lists the various hydrocarbon products. In the second column, hydrocarbons with three or more carbon atoms (C_3^+), were determined by a summation of components of a complete analysis; in column three, gases were determined by type analysis.

Table II. Hydrocarbons in Automobile Exhaust

(1000 r.p.m. no load)

Hydrocarbon	Complete Analysis	Type Analysis
Methane	0.07 ± 0.02[a]	
Acetylene	0.04 ± 0.02	
Ethylene	0.07 ± 0.02	
Ethane	0.05 ± 0.02	
Total cracked hydrocarbons	0.23 ± 0.05	
C_3^+ paraffins	0.06[b] ± 0.02	0.05 ± 0.01
C_4^+ paraffins	0.05 ± 0.02	0.05 ± 0.02
C_3^+ olefins	0.09 ± 0.02	0.08 ± 0.02
C_5^+ olefins	0.07 ± 0.02	0.10 ± 0.04
C_4^+ CODA[c]		0.03 ± 0.01
C_3^+ CODA	0.03 ± 0.01	0.03 ± 0.01
Aromatics	0.05 ± 0.03	0.06 ± 0.03
Total gasoline	0.23 ± 0.06	0.22 ± 0.06
Total hydrocarbons	0.46 ± 0.08	0.45 ± 0.08

[a] Maximum deviation from mean of twelve analyses.
[b] Summation of individual hydrocarbons.
[c] Abbreviation for cyclo-olefins, diolefins, and acetylenes.

Results of these analyses indicate that the hydrocarbons in exhaust gases emitted from a car with engine idling, under these particular conditions, are approximately 50% gasoline and 50% cracked gasoline products. This does not take into consideration the products from the low vapor pressure lubricating oils. The C_3^+ and C_5^+ components of the analysis agree within the maximum order of uncertainty; therefore, the difference does not appear to be significant at this concentration level.

CONTINUOUS ANALYSIS

Since the collection of representative exhaust gas samples is difficult and the intermediate products may no longer be present on final analysis, it seemed advisable immediately to monitor the gases of interest at various engine speeds. A portable mass spectrometer was connected directly to the automobile and the significant spectral peaks were recorded.

Figure 2 presents a family of curves derived from the total recorded peak intensities on the monitor mass spectrometer *vs.* engine revolutions per minute. Mass 2, indicative of the hydrogen content of the exhaust gas, reaches a maximum at 1000 revolutions per minute. This is presumed to be the condition of engine performance most conducive to cracking of hydrocarbons and reaction of the combustion products—i.e., the water gas reaction:

$$CO + H_2O \rightleftharpoons H_2 + CO_2$$

The air content of the exhaust, indicated by the *m/e* 32 curve, does not reach a maximum until 1500 revolutions per minute, a function of carburetion. The general trend of hydrocarbon content in the exhaust is represented by *m/e* 26, 27, 41, and 42. These gases appear to reach a maximum at 2000 revolutions per minute. The products of the atmosphere, nitrogen and argon,

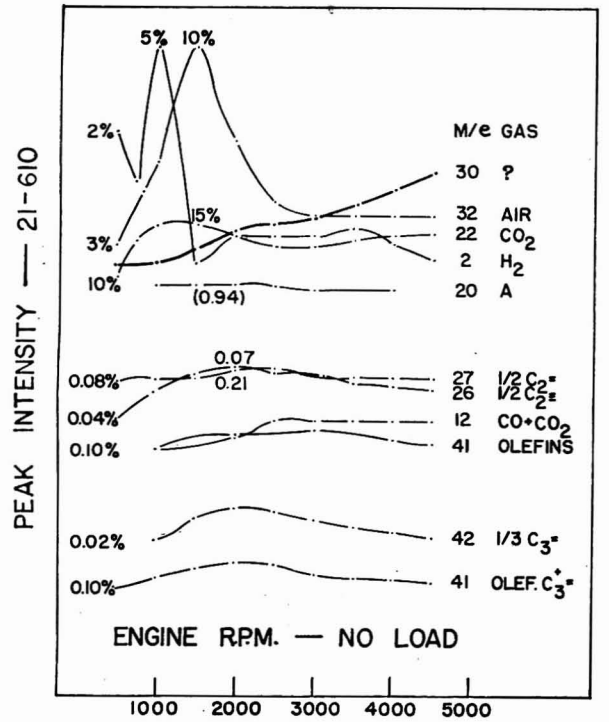


Figure 2. Monitored mass spectrometer spectral peaks

show little variation. The *m/e* 22 curve denotes carbon dioxide. The *m/e* 12 curve is a result of both carbon monoxide and carbon dioxide.

The most significant effect noted was the curve shown by *m/e* 30. In previous analyses, excess *m/e* 30 over and above $C^{12}O^{18}$ and N_2^{15} was considered ethane. No differentiation could be made among formaldehyde, nitric oxide, and ethane. The additional sensitivity of the monitoring mass spectrometer reveals a steady increase in the *m/e* 30 intensity with engine revolutions per minute; this value returns to the original intensity upon deceleration. Since all other hydrocarbon, nitrogen, and oxides of carbon peaks tend to decrease or to reach a steady state at higher values of revolutions per minute, the entire peak probably does not result from these gases. The two remaining possibilities are aldehydes (HCHO+) or oxides of nitrogen (NO+).

Aldehydes in general are very easily oxidized to acids (*3*). It is therefore unlikely that more aldehydes would be produced under the most extreme conditions of oxidation. However, the equilibrium of the reaction

$$N_2 + O_2 \rightleftharpoons 2NO$$

shifts to the right with increasing temperature. NO, the most stable oxide of nitrogen, remains the most likely gas present. This fixation produces less than 1% nitric oxide at 1500° C. which slowly oxidizes to nitrogen dioxide on cooling to 600° C. (*6*). Both of these oxides are water-soluble. This reaction is also used to produce nitric acid commercially by the Birkeland-Eyde process in Norway.

SAMPLING PROCEDURE

Numerous sampling techniques were tried and found to vary considerably with volume, time, and apparent differential flow of the exhaust gas constituents. Reproducibility of analyses and sampling can be achieved without obtaining theoretical amounts of water. A 3-foot length of air cooled aluminum tubing was sufficient to effect complete water condensation ahead of the sample container. Pumping of the gas through the container will result in gas fractionation (*2*).

Results of three analyses are compared in Table III. Column 1 shows an analysis of the hydrocarbon and water-soluble gases in a mixture containing theoretical water. Column 2 shows an average of four analyses containing 0.36% water and column 3 presents a sample containing excessive water. These results indicate that the condensation of water during sampling is accompanied by a decrease in gasoline and water-soluble gases. Similarly, higher operating and sampling temperatures must be used to study the oil vapor content of exhaust gases (*8*).

Special 1-ml. brass sample containers fitted with needle valves were used in sample collection. These containers, shown in Figure 3, were attached directly to the exhaust manifold of the car by means of a threaded sampling tube welded into the line. The sampling tube, cut in cross section, was equal in length to the radius of the manifold. The containers were allowed to reach full manifold temperature (10 minutes) before the samples were removed for immediate introduction to the mass spectrometer. The sample containers were heated by torch while the samples entered the instrument inlet system.

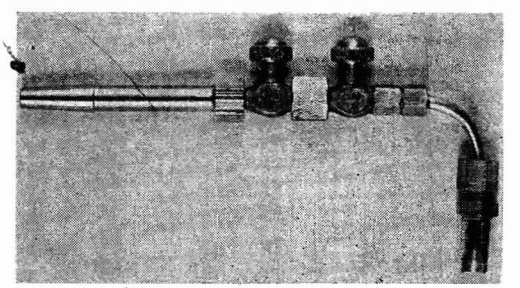


Figure 3. Brass container with needle valve for sample collection

Table III. Variation in Gasoline and Water-Soluble Gases with Total Water Content

Gas	1	2	3
$<C_3$ hydrocarbons	0.22	0.20	0.27
$>C_4$ gasoline	0.22	0.07	0.51
Water	14.4	0.36	30.0
Alcohols	0.01	..	0.02
Sulfur dioxide	0.07	..	0.20
Hydrogen sulfide	0.01	..	0.03

INSTRUMENTATION

The laboratory gas analyses were performed in a standard Consolidated mass spectrometer, Model 21-103B (*12, 13*). The entire mass spectrometer inlet system was flamed with an air-gas torch and allowed to pump out prior to analyses. The 1-ml. sample container was attached to the inlet system through a Teflon tapered gasket to eliminate the use of stopcock grease. The entire sample and line were heated to approximately 100° C. and expanded into the reservoir, where the final pressure was found to be 150 microns of mercury as measured on the micromanometer. The measured pressure was used as an index of total condensable products present, to indicate the sample was representative.

Continuous analyses were performed on a standard Consolidated monitor mass spectrometer, Model 21-610 (*4*). The sample line from the exhaust manifold was connected directly to the instrument (Figure 1). A Sun Dwell meter was used to indicate engine revolutions per minute *vs.* peak intensities.

SPECTRAL INTERPRETATION

The gas samples were scanned from mass 2 to 200. Peaks were found through the C_{10} aromatics at *m/e* 134. Typical spectra are

Table IV. Mass Spectra Interpretation of Complete Analysis

m/e	Component Gas	API Serial No.
2	Hydrogen	2[a]
12	Carbon monoxide	156[a]
14	Nitrogen	[a]
15	Methane	1[a]
18	Water	[a]
20	Argon	[a]
22	Carbon dioxide	157[a]
26	1/2 Acetylene	72[a]
27	1/2 Ethylene	23[a]
30	Ethane	2[a]
31	Alcohols	Average 363, 364, 366
32	Air	[a]
42	1/2 Propylene	24[a]
43	1/2 C_3-C_5 paraffins	[a]
48–64	Sulfur dioxide	97
55	C_4-C_5 olefins	[a]
57–71	Heptanes	19
78	Benzene	250[a]
81	Hexyne	532
82	Hexyne	433
83	Heptenes	977
84	Hexenes	106
85	Octanes	51
92	Toluene	251[a]
97	Octenes	131
104	Styrene	[a]
105–120	Cumene	257[a]
106	Xylene	255[a]
111	Nonenes	228
118	Methylstyrenes	[a]
134	C_{10} aromatics	[a]

[a] Calibrations run in laboratory.

Table V. Mass Spectra Interpretation of Type Analysis

m/e	Component Type	Peaks Used
	Sensitivity factors derived from (1)	
Σ43	C_3^+ paraffins	43, 57, 71, 85, 99, 113, 127
Σ71	C_5^+ paraffins	71, 85, 99, 113, 127
Σ41	C_3^+ olefins	41, 55, 69, 83, 97, 111, 125
Σ69	C_5^+ olefins	69, 83, 97, 111, 125
Σ53	C_4^+ CODA[a]	53, 54
Σ67	C_5^+ CODA	67, 68, 81, 82, 95, 96, 109, 110, 123, 124
Σ77	Aromatics	77, 78, 79, 91, 92, 105, 106, 119, 120, 133, 134

[a] Abbreviation for cyclo-olefins, diolefins, and acetylenes.

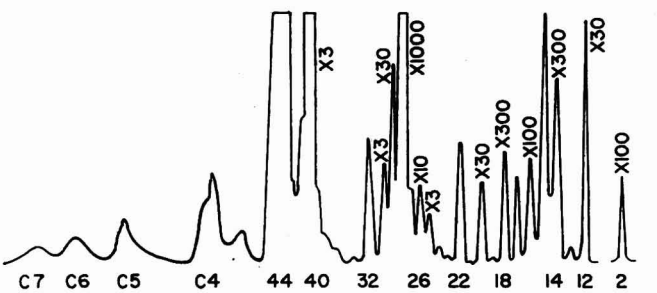


Figure 5. Scanned spectra of exhaust gas at engine speed of 2550 r.p.m.

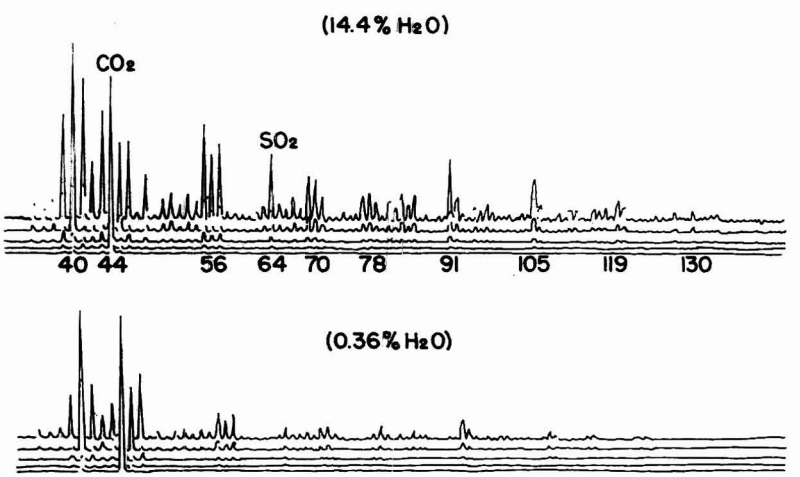


Figure 4. Spectra of gasoline in exhaust gas

shown in Figure 4. Complete analyses were accomplished by a material balance of peak intensities *vs.* specific API spectra as outlined in Table IV. A similar simplified computing procedure was followed as indicated in column 2. Many of the calibrations for the compounds listed were available in the laboratory. Results of these analyses were then compared to those determined by gasoline-type analysis (*1*).

This method was modified slightly as indicated in Table V, in order to determine C_3 and C_4 cracked hydrocarbons if present. In this determination Σ43 plus Σ71, Σ41 plus Σ69, and Σ53 plus Σ67 were used in addition to Σ71, Σ69, Σ67, and Σ77. Because the above method is limited to gasoline analyses containing C_4 to C_{15} hydrocarbons, the difference between C_3^+ ions and C_5^+ ions should indicate the quantity of C_3 and C_4 cracked products. This procedure should differentiate the gasoline from the cracked products. The actual differences determined (Table II) were of the same order as the uncertainty and considered insignificant.

Although the Model 21-610 mass spectrometer lacks the resolution necessary for the analytical treatment described in Table IV, in the gasoline range, the results have shown that *m/e* 26 is approximately 50% acetylene; the *m/e* 27, 50% ethylene; the *m/e* 41, 40% unsaturated gasoline; and the *m/e* 43, 45% saturated hydrocarbons from gasoline. A similar more complete treatment can be made using the spectral summation of the C_4, C_5, C_6, and C_7 ions in spectra scanned at an engine speed of 2550 r.p.m. shown in Figure 5.

CONCLUSION

Results of this limited investigation of the composition of automobile exhaust gases indicate the importance of proper sampling procedures. Total water content of the gas was used as a measure of representative sampling. By continuous recording mass spectra at various engine speeds, changes in exhaust gas composition can be demonstrated. The hydrocarbon and air content of exhaust approaches a steady state at high engine speeds, while the oxides of nitrogen increase proportionately with engine speed and temperature.

ACKNOWLEDGMENT

The authors wish to thank Henry Landsberg and Stanley Dawkins for their assistance and cooperation in running the 21-610 mass spectra on the automobile exhaust gases.

LITERATURE CITED

(1) Brown, R. A., ANAL. CHEM., 23, 430 (1951).
(2) Fluke, Donald, *Rev. Sci. Instr.*, 19, 665 (1948).
(3) Gilman, Henry, and coworkers, "Organic Chemistry," Vol. I, p. 655, Wiley, New York, 1949.
(4) Haagen-Smit, A. J., Bradley, C. E., and Fox, M. M., "Proceedings of Second National Air Pollution Symposium," p. 55, Pasadena, Calif., May 5 and 6, 1952.
(5) Magill, P. L., Hutchison, D. H., and Stormes, J. M., *Ibid.*, p. 71.
(6) Mellor, J. W., "Mellor's Modern Inorganic Chemistry," p. 406, Longmans, Green and Co., London, 1939.
(7) Obert, E. F., "Internal Combustion Engines," p. 308, International Textbook Co., Scranton, Pa., 1950.
(8) O'Neal, M. J., Jr., and Wier, T. P., Jr., ANAL. CHEM., 23, 830 (1951).
(9) Rock, S. M., *Ibid.*, 23, 261 (1951).
(10) Shepherd, M., Rock, S. M., Howard, R., and Stormes, J. M., *Ibid.*, 23, 1431 (1951).
(11) Walker, J. K., Gifford, A. P., and Nelson, R. N., *Ind. Eng. Chem.*, 46, 1400 (1954).
(12) Washburn, H. W., Wiley, H. F., and Rock, S. M., IND. ENG. CHEM., ANAL. ED., 15, 54 (1943).
(13) *Ibid.*, 17, 74 (1945).

RECEIVED for review August 30, 1954. Accepted January 3, 1955.

Electrostatic Sampler for Dust-Laden Gases

G. L. ROUNDS and H. J. MATOI

Air Control and Research, Kaiser Steel Corp., Fontana, Calif.

An electrostatic sampler has been designed, which will collect up to five times the weight of dust collected by conventional electrostatic samplers. The device uses a commercially available high voltage source. The collecting surface is in the form of a truncated cone for more uniform dust deposition. The unit can be disassembled in a few seconds for cleaning.

IN THE process of accurately sampling a gaseous mixture for both gaseous and solid constituents, one of the most important considerations is the selection of the device that will provide the initial separation of the gases and the solids. This selection is dependent upon the subsequent analytical treatment to which the sample is to be subjected. The following treatments and requirements are considered important in the field of air pollution.

A. Dust loading of gas stream. A highly efficient device.
B. Chemical determination of the elements in their native state of oxidation in the gaseous and solid phases. A device that subjects the sample to neither an oxidizing nor reducing atmosphere.
C. Complete chemical analyses of the two phases. A device that will not contaminate sample or react with it.
D. Analyses of the gaseous portion for compounds likely to adsorb on the surface of the particulate. A device capable of performing at the temperatures existing in the native gas-solid mixture, and which provides minimum contact between the gaseous and solid phases.
E. Chemical determination of elements or compounds that occur in both phases. A highly efficient device, which provides minimum contact between the two phases, and is capable of performing at the temperatures existing in the native gas-solid mixture.
F. Particle size determination. A nonagglomerating, nonfracturing, and highly efficient device.

The most common type of apparatus for the separation of the particulate from the gaseous phase incorporates a paper filter upon which the solid is deposited and through which the gases pass. Several of these devices perform well (*4*). Paper filters, however, are subject to specific limitations (*2*) and meet only requirements A and B. The paper filter agglomerates the dust, has low temperature limits, and contaminates the particulate sample with organic material.

The paper filter also produces a rather high pressure drop, which often seriously limits the rate at which the sample can be taken. This limitation often makes isokinetic sampling of stack gases difficult. If water is allowed to condense on the filter paper, the gaseous phase is subjected to an undesirable scrubbing action. Condensation of water also causes very high pressure drops, which weaken the filter and increase the tendency to rupture. The determination of the amount of collected dust on the filter paper presents a problem that has not been solved to the satisfaction of this laboratory.

The paper of the filter absorbs some of the gaseous compounds and thus provides a false separation of the gaseous and solid states of a particular element, especially if very low concentrations are encountered. The paper filter, however, does provide a most convenient and expeditious method of separating the gaseous and solid phases.

Because of the limitations of the paper filter, this laboratory designed a sampler that operates on the electrostatic principle. This sampler was based on a combination of theory and practice. Equations and references are listed by Perry (*3*), but it was found that the final design was best reached by the process of trial and error, because of the numerous variables imposed by the changing chemistry of the dust and gas encountered during stack gas sampling. This equipment was designed in 1953 and subsequent testing has shown it to be applicable to requirements A and C and, providing appropriate insulators are used, to D and E.

The Mine Safety Appliances Co., Pittsburgh, Pa., has an electrostatic sampler which is designed for sampling the atmosphere, but makes no provision for sampling stack gases. The Western Precipitation Corp., Los Angeles, Calif., manufactures an electrostatic stack gas sampler called the "sampling electrofilter." However, this unit incorporates a cylindrical collecting tube and is thus restricted to a dust-loading limit of from 1 to 2 grams. Furthermore, the Western unit incorporates a porcelain insulator which is subject to attack by open hearth gases.

The electrostatic sampler designed by the authors has a very low pressure drop at the rated flow of 1.5 cubic feet per minute or less. The device is constructed of stainless steel, which minimizes reaction with the stack gases and allows a wide range in sampling temperatures. The collected dust sample can be quantitatively recovered from the inside walls of the sampling tube with no contamination from the sampler.

As a power source, the standard Mine Safety Appliance "power pack" used on the M.S.A. electrostatic sampler has been found very reliable. Using this source, it is possible to obtain approximately 15 kv. across the precipitator, depending on the relative humidity of the gases being sampled and the electrical properties of the dust being collected. Fluctuations in the 110-volt power source cause significant variations in the output kilovoltage, which necessitate fairly frequent observation and readjustment of the power pack output.

All of the stack gases and atmospheric samples tested, to date, have been quantitatively cleaned of the particulate material by 13 kv. impressed across the precipitator. This potential also allows some leeway for increasing the output in the event of a drop in the 110-volt power source.

Figure 1 portrays the sampling setup, including a pair of plastic impingers designed by the authors, and Figures 2 and 3 show the construction of the sampler.

The gas enters through the tangentially positioned 1/4-inch pipe at the lower end of the collecting tube. Any very large pieces of solid material are separated by the slight centrifugal action and are found resting on the bottom plate at the completion of the sample.

The collecting tube is in the form of a truncated cone, in order to afford a more uniform distribution of dust in the bottom third to half of the tube. As the dust progresses upward through the

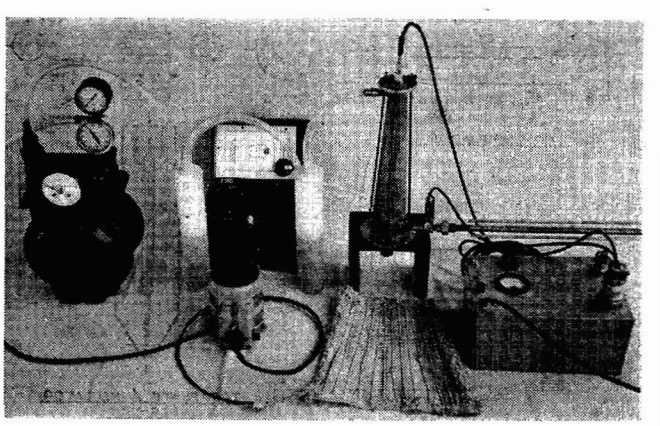


Figure 1. Stack sampling setup

tube, it is subjected to an electrostatic field of increasing intensity. Because of the small diameter of the exit end of the sampler, the field intensity has reached the point of breakdown in normal atmosphere at approximately 20 kv. Cylindrical tubes were found to provide very thick dust deposition in the lower fourth of the tube, resulting in excessive blowoff after approximately 1 gram was collected, and thus seriously reducing the sample volume for which high efficiency was afforded. Many samples of open hearth effluent have been taken with excellent efficiencies, when as much as 10 grams were collected in the truncated cone collecting tube.

The negative discharge electrode consists of a 0.027-inch stainless steel wire, fixed to the top insulator (Figure 3) by means of the stainless steel bushing incorporating a side setscrew. The bottom insulator has a 0.032-inch blind hole drilled 1/2 inch deep, which provides a support for the free end of the electrode.

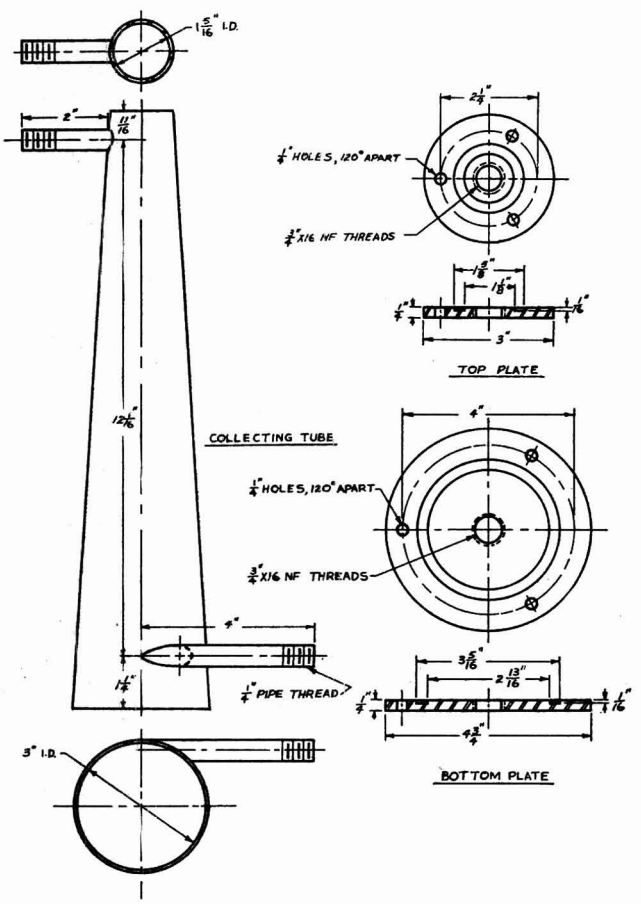


Figure 2. Collecting tube and plates

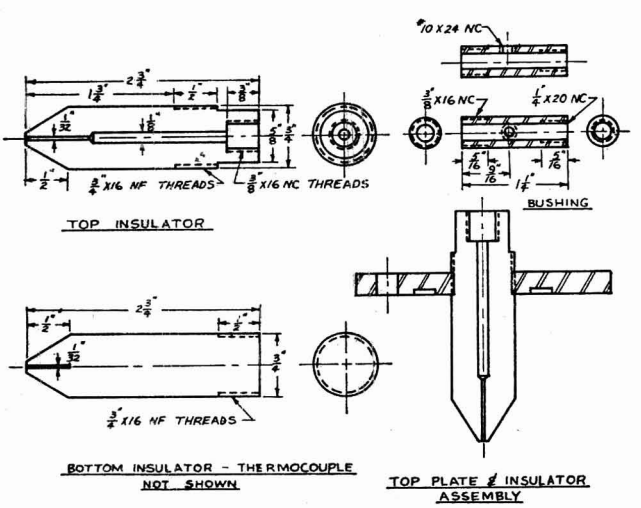


Figure 3. Insulators and bushing

Electrically, Teflon is ideal for its application here, in that its dielectric strength in the presence of a corona is 50 to 75 volts per 0.001 inch. Its electrical properties do not change over the wide temperature range of −100° to +500° F., and its surface resistivity is high at high humidities. It was because of these properties as well as its inertness to corrosive gases, that Teflon was chosen for the insulators; however, many ceramic materials will suffice when the gases sampled do not contain fluorides. An important consideration in the choice of an insulator material is its dielectric properties, to withstand the high potential.

A single insulator unit was constructed and tests proved that the free end of the electrode was subject to excessive oscillation, resulting in arcing and low efficiencies. The lower insulator is provided with a small platinum-rhodium thermocouple entering from the lower end of the insulator. The thermocouple hot junction is exposed through the side of the insulator at the point of entrance of the gases. The temperature of the inlet gases is thus determined by use of a conventional potentiometer. The incorporation of a thermocouple within this insulator is important because of the tendency of the Teflon (polytetrafluoroethylene) insulators to decompose. If fluorides are to be determined in the solids and gases collected, the temperature of the inlet gases must be kept below 350° F. However, if a slight amount of gaseous emissions from the insulators can be coped with, the minimum gas inlet temperature can be raised to 500° F. Other temperature properties of Teflon as listed by the Graef Engineering Co., Paramount, Calif., are its change to a transparent gel at about 620° F. and its decomposition into gaseous constituents at approximately 750° F. Teflon will not support combustion.

An electric heating jacket was designed in order to maintain the desired temperature within the precipitator and is shown unmounted in Figure 1. If stack gases are being sampled, it is necessary to maintain temperatures above the dew point. Normally this temperature falls between 100° and 200° F. At these temperatures the life of the insulator should be indefinite.

Open hearth effluent is considered to have many undesirable electrostatic precipitating characteristics; however, several hundred samples of this effluent have been taken with this sampler and excellent efficiencies have resulted. To test the efficiency of dust separation of the sampler, a holder incorporating an 11.0-cm. filter paper (Whatman No. 32 or 42) was placed on the exit of the precipitator. These papers are extensively used in flat filter holders for filtering dust-laden gas streams, because of their tight structure (*2*). In only a few cases was there any observable red color due to the iron oxide dust added to the paper, and in no case could the addition to the paper be detected on a laboratory analytical balance. The open hearth effluent upon which these efficiency studies were made consisted of materials collectable on Whatman No. 32 or 42 papers. Approximately 85% of this material was under 5 microns in diameter.

All electrostatic precipitators have the disadvantage of producing oxides of nitrogen and ozone. Perry (*3*) notes that ozone formation is less from a positive discharge electrode than from a negative discharge. Beadle, Kitto, and Blignaut (*1*) state that negative ionization produces 10 times as much ozone as positive ionization. However, negative ionization occurs at a lower applied potential and generally has a higher sparking potential and less erratic behavior, resulting in better collection efficiencies. It appears that positive ionization has only the advantage of lower ozone production, which should be considered when the presence of large amounts of ozone is not desirable.

The effect of the generated oxidizing agents on the gas-solid mixture to be sampled should be carefully evaluated before electrostatic methods are used. To date, no deleterious effects have been detected in open hearth stack sampling, although this oxidizing interference is probably present when a mixture of air and organic gases is sampled. The use of electrostatic methods involving mixtures of combustible substances and air should be avoided.

LITERATURE CITED

(1) Beadle, D. G., Kitto, P. H., and Blignaut, P. J., *Arch. Ind. Hyg. and Occupational Med.*, **10**, 381–9 (1954).
(2) Chambers, L. A., *Am. Ind. Hyg. Assoc. Quart.*, **15**, 290–6 (December 1954).
(3) Perry, J. H., "Chemical Engineers' Handbook," 3rd ed., pp. 1039–45, McGraw-Hill, New York, 1950.
(4) Stanford Research Institute, Stanford, Calif., private correspondence.

RECEIVED for review September 20, 1954. Accepted February 7, 1955.

Two-Step Mixed Indicator for Kjeldahl Nitrogen Titration

IRVING H. SHER[1]

Department of Chemistry, The Mount Sinai Hospital, New York 29, N. Y.

Bromocresol green, new coccine, and *p*-nitrophenol are used as a mixed indicator in Kjeldahl nitrogen titration.

WHEN variations of the micro-Kjeldahl nitrogen determination are employed, which call for distillation of the ammonia, current usage seems to favor the method proposed by Winkler (*9*), in which the ammonia is trapped in a boric acid–indicator solution and titrated with a standardized mineral acid. Indicators, such as bromophenol blue, methyl orange, Congo red, and alizarin red S, have proved generally unsatisfactory for this titration. Methyl red and bromocresol green are, perhaps, the indicators most frequently employed, but either, when used alone, has an end point that calls for careful comparison with a standard. The methyl red changes from reddish yellow (alkaline) to yellowish red (acid) at about pH 5.2, while bromocresol green goes from bluish green (alkaline) to yellowish green (acid) at about pH 4.6.

Several mixed indicators have been devised in efforts to make the ammonia end point more discernible to the eye. The term "mixed indicator," as used here, includes mixtures of actual indicators as well as mixtures of indicators with constant-background dyes. Such a mixture is methyl red–methylene blue (*1*, *7*), which has a gray end point at pH 5.3, is greenish gray in more alkaline solutions, and is purplish gray in more acid solutions. Methyl red–bromocresol green, which has also been used in various proportions (*3*, *5*, *6*), changes less sharply at about pH 4.6 from bluish green or blue-gray (alkaline), according to the relative amounts, to pink or violet-red (acid) without going through neutral gray. Methyl red–tetrabromophenol blue is the best of several indicator mixtures tested by Stover and Sandin (*8*); it changes from green (alkaline) through gray to pale gray-violet (acid). Nutten (*4*), in a review of new indicators, speaks of a methyl red–alphazurine mixture, which changes from a greenish gray at pH 4.8 to a purplish gray at 4.6. The best combinations for the ammonia titration, as reported by Johnson and Green (*2*), were (1) methyl red mixed with methylene blue or guinea green, which changed from green (alkaline) to violet (acid) at pH 5.2, and (2) alizarine red S mixed with indigo carmine or guinea green, which changed from violet (alkaline) to green (acid) at pH 5.2.

It has been found here that bromocresol green may be used as the basis for a mixed indicator which has high sensitivity, an easily discernible color change, a neutral gray end point, and a good warning signal toward the end of the titration. In its transition range (pH 5.4 blue to 3.8 yellow) bromocresol green itself is sensitive, giving different colors with changes of less than 0.1 pH unit. A relatively constant green color, however, partly obscures the effect. The addition of new coccine (a dye manufactured by Ansco, Binghamton, N. Y., which can be purchased at most photographic supply stores) in the proper proportion will cancel this green, leaving a light gray background. A very sharp end point then occurs, changing, upon the addition of 0.01 ml. of 0.01429*N* hydrochloric acid, from blue (alkaline) to gray and then to yellow (acid) with a second addition of 0.01 ml. The introduction of *p*-nitrophenol to the mixture does not affect this end point, as the former is colorless at this pH, but does contribute a yellow color in the more alkaline region. The color changes of this bromocresol green–new coccine–*p*-nitrophenol indicator then occur as given in Table I. The gray end point at pH 4.6 is easily seen, is sharpest by fluorescent light or daylight, and requires no standard for comparison. The amount of ammonium chloride formed from 1 mg. (≈5 ml. of 0.01429*N* hydrochloric acid) buffers the solution only to the extent that an additional 0.021 ml. of acid is required to bring the solution from pH 5.2 (the equivalence point for the ammonium chloride) down to pH 4.6. During an actual titration of micro amounts of ammonia in boric acid, the colors and shades change continuously below pH 7.4 and the distinctive green to blue warning occurs at about 0.5 ml. of 0.01429*N* hydrochloric acid before the gray end point. This, of course, facilitates more rapid titrations. None of the other indicators mentioned above can use to advantage the alkaline yellow contribution of *p*-nitrophenol, as most of them are yellow, green, or violet on the alkaline side of their end points. The mixed indicator of Reith and Klazinga (*5*), which consists of 1 part of methyl red and 3 parts of bromocresol green, can be given a secondary color change by adding 9 parts of *p*-nitrophenol. This mixture will turn from green to grayish blue to reddish violet as acid is added, but neither transition is as sharp as with the bromocresol green–new coccine–*p*-nitrophenol indicator.

INDICATOR

Preparation. Dissolve 350 mg. of bromocresol green in 10 ml. of 95% ethyl alcohol in a 250-ml. volumetric flask. Mix in 1.0 ml. of 0.50*N* sodium hydroxide and add about 200 ml. of distilled water.

Add 22.1 ml. of an aqueous 1% solution of new coccine and then add 750 mg. of *p*-nitrophenol, which has been dissolved in a few milliliters of 95% ethyl alcohol. Dilute to 250 ml. with distilled water.

Table I. Color Changes of Bromocresol Green–New Coccine–*p*-Nitrophenol Indicator in 0.5*M* Phthalate Buffer Solutions

pH	Color
>7.6	Green
7.4	Bluish green
6.6	Deep blue
5.7	Blue
4.8	Grayish blue
4.7	Bluish gray
4.6	Gray
4.5	Yellowish gray
4.4	Grayish yellow
<4.4	Yellow to yellow orange

Test a few drops of the indicator in an acetate or phthalate buffer at pH 4.6. If the light gray color is not completely neutral as seen by the type of light to be used in subsequent titrations, add small known amounts of new coccine solution or bromocresol green solution to 1-ml. portions of the indicator and retest with the buffer. When the color is neutral gray, correct the bulk of the indicator by a proportionate amount.

Use. For good sensitivity dilute 15 ml. of this stock indicator containing 40 grams of C.P. boric acid to 2 liters with distilled water. Use 5 ml. of this boric acid–indicator mixture in the usual way to trap ammonia from the distillation. Handle a blank in the same manner and, before the titration, bring all samples to 25 ml. with ammonia-free water.

Errors due to increased volumes during titrations up to 5 ml. (≈1 mg. of nitrogen) amount to only about 0.1%. The volume of stock indicator used may be halved or doubled according to

[1] Present address, Research Laboratories, The National Drug Co., Philadelphia 44, Pa.

personal preference, and according to the shape of the vessel in which the titration is done.

LITERATURE CITED

(1) Brecher, C., *Wien. klin. Wochschr.*, 49, 1228 (1936).
(2) Johnson, A. H., and Green, J. R., IND. ENG. CHEM., ANAL. ED., 2, 2 (1930).
(3) Ma, T. S., and Zuazaga, G., *Ibid.*, 14, 280 (1942).
(4) Nutten, A. J., *Metallurgia*, 42, 216 (1950).
(5) Reith, J. F., and Klazinga, W. M., *Chem. Weekblad.*, 38, 122 (1941).
(6) Sobel, A. E., Mayer, A. M., and Gottfried, S. P., *J. Biol. Chem.*, 156, 355 (1944).
(7) Sobel, A. E., Yuska, H., and Cohen, J., *Ibid.*, 118, 443 (1937).
(8) Stover, N. M., and Sandin, R. B., IND. ENG. CHEM., ANAL. ED., 3, 240 (1931).
(9) Winkler, L. W., *Z. angew. Chem.*, 26, 231 (1913).

RECEIVED for review July 19, 1954. Accepted January 4, 1955. Work carried out under grant C-1791 from United States Public Health Service.

Detection of Gallium with Rhodamine B

HIROSHI ONISHI

School of Chemistry, University of Minnesota, Minneapolis, Minn.

A sensitive color and fluorescence reaction of gallium with rhodamine B is described. Interference by antimony, gold, iron, thallium, and tungsten is eliminated by reducing these ions with titanous chloride. As little as 0.01 γ of gallium can be detected by the orange-yellow fluorescence of the benzene extract of rhodamine B chlorogallate in ultraviolet light, and 0.1 γ by a red-violet color.

RHODAMINE B is a sensitive color and fluorescence reagent for antimony (*3, 8*), gold (*4*), thallium (*5*), and tungsten (*3*). Apparently its reaction with gallium has been overlooked. Traces of gallium can be detected by adding rhodamine B to a test solution which is 6*M* in hydrochloric acid and shaking with benzene. In the presence of gallium, the benzene layer shows a red-violet color and an orange-yellow fluorescence in ultraviolet light. In sulfuric acid solution free from chloride no reaction is observed. This suggests that the reaction product is rhodamine B chlorogallate. The solubility of this product in 6*M* hydrochloric acid is much greater than that of the rhodamine B chloro complexes of the other metals, but its benzene extraction is successful.

Of the various gallium reactions available at the present time (*1, 2, 6*), that with 8-quinolinol (*7*) is the most selective, but it requires the use of a buffered solution.

REAGENTS

Rhodamine B, 0.5 gram in 100 ml. of 6*M* hydrochloric acid.

Titanous chloride, 20%. Purify by washing 30 ml. of the solution with two 10-ml. portions of isopropyl or diethyl ether (peroxide-free).

Hydrochloric acid, 1 to 1.

PROCEDURE

A. In Absence of Antimony, Gold, Iron, Thallium, and Tungsten. Adjust the acidity of the solution (volume conveniently ~2 ml.) to 6*M* in hydrochloric acid. Oxidizing agents, including nitrate, should be absent. Transfer the solution to a small glass-stoppered tube. Add 0.4 ml. of rhodamine B solution, mix, and shake for 1 minute with 2 ml. of benzene. Allow to stand for several minutes (or centrifuge) to allow droplets of water to separate from the benzene phase. A pink to red-violet color in the benzene indicates the presence of gallium. Fluorescence may be observed in ultraviolet light. When minimal amounts (less than 0.5 γ) of gallium are being tested for, the solution should be compared against a blank.

B. In Presence of Ions Mentioned Above. Add 0.5 ml. of titanous chloride solution (or the minimum amount required for reduction of ferric iron and any other reducible substances) to the test solution, which is 6*M* in hydrochloric acid. As much as 20 mg. of iron(III) may be present. Allow to stand for about 5 minutes and then proceed as described above. Centrifugation is necessary.

SENSITIVITY

Sensitivities obtained (~2 ml. of aqueous phase, 2 ml. of benzene) are summarized in the following table.

	Procedure A		Procedure B	
	Color	Fluorescence	Color	Fluorescence
Limit of identification, γ gallium	0.05	0.01	0.01	0.01
Dilution limit, in benzene (or aqueous phase)	$1:4 \times 10^7$	$1:2 \times 10^8$	$1:2.5 \times 10^7$	$1:2 \times 10^8$

Under the conditions specified about one third of the gallium is extracted into the benzene phase.

EFFECT OF OTHER IONS

By Procedure B it is possible to detect 0.5 γ of gallium in the presence of 20 mg. of iron, 10 mg. of antimony, 1 mg. of thallium, and 0.1 mg. of gold. In the absence of gallium the blank benzene solution is practically colorless. Vanadate (1 mg. of vanadium) and tungstate (1 mg. of tungsten) do not give colors in the benzene phase and do not interfere with the detection of 0.5 γ of gallium.

The following ions give no color with rhodamine B under the conditions (Procedure B) and do not interfere in the detection of as little as 0.5 γ of gallium:

Li^+ (10 mg.), Na^+ (10 mg.), K^+ (10 mg.), Cu^{++} (10 mg.), Ag^+ (0.1 mg.), Be^{++} (1 mg.), Mg^{++} (10 mg.), Ca^{++} (10 mg.), Sr^{++} (10 mg.), Ba^{++} (10 mg.), Zn^{++} (10 mg.), Cd^{++} (1 mg.), Hg^{++} (1 mg.), Al^{+++} (100 mg.), In^{+++} (1 mg.), Sc^{+++} (1 mg.), Y^{+++} (1 mg.), Ge^{4+} (1 mg.), Sn^{++} (10 mg.), Pb^{++} (10 mg.), Zr^{4+} (1 mg.), P^{5+} (as Na_2HPO_4, 10 mg.), As^{+++} (1 mg.), Bi^{+++} (1 mg.), Ta^{5+} (1 mg.), Se^{4+} (1 mg.), Te^{4+} (1 mg.), Cr^{+++} (10 mg.), Mo^{6+} (1 mg.), F^- (10 mg.), I^- (10 mg.), Mn^{++} (10 mg.), Co^{++} (10 mg.), Ni^{++} (10 mg.), and Pt^{4+} (1 mg.).

Sulfuric acid—e.g., 1*N*—does not interfere. Gallium is readily detected in the presence of 200,000 times as much aluminum.

The sensitivity of the test can be increased by first concentrating gallium by ether extraction from 6*M* hydrochloric acid solution. This also removes antimony, gold, iron, thallium, tungsten, and most other elements if a suitable reducing agent—e.g. titanous chloride—is present. Procedure A may then be applied.

ACKNOWLEDGMENT

The author wishes to express his appreciation to E. B. Sandell for advice and suggestions.

LITERATURE CITED

(1) Beck, G., *Mikrochim. Acta*, 2, 287 (1937).
(2) Charlot, G., *Anal. Chim. Acta*, 1, 218 (1947).
(3) Eegriwe, E., *Z. anal. Chem.*, 70, 400 (1927).
(4) Feigl, F., "Spot Tests," Vol. I, "Inorganic Applications," 4th English ed., p. 124, Elsevier Press, Houston, 1954.
(5) Feigl, F., Gentil, V., and Goldstein, D., *Anal. Chim. Acta*, 9, 393 (1953).
(6) Patrovský, V., *Chem. Listy*, 48, 537 (1954).
(7) Sandell, E. B., IND. ENG. CHEM., ANAL. ED., 13, 844 (1941).
(8) West, P. W., and Hamilton, W. C., ANAL. CHEM., 24, 1025 (1952).

RECEIVED for review November 8, 1954. Accepted January 24, 1955

A Study of Oxidations Using Copper(III) Reagents

DONALD A. KEYWORTH[1] and K. G. STONE

Kedzie Chemical Laboratory, Michigan State College, East Lansing, Mich.

The determination of organic compounds on a micro scale by oxidation with potassium diperiodato cuprate (III) and potassium ditellurato cuprate(III) in alkaline media was reported by Beck. Reagent solutions are easy to prepare and appear to be stable but no good method could be found for end-point detection. Data are presented to show that periodate and tellurate ions participate in the oxidation of many compounds, but reproducible results are difficult to obtain. The oxidation of cyanide ion and thiosulfate ion is almost quantitative. New methods employing copper(III) reagents were studied to demonstrate the usefulness of the application.

THE use of potassium diperiodato cuprate(III) and potassium ditellurato cuprate(III) as volumetric reagents has been described by Beck on a micro scale (*3, 4*) and especially for biochemical applications (*1, 2*). The results suggest that an evaluation of the reagents at macro concentration levels should be made.

Compounds containing copper(III) have been known since 1844, and the early work has been reviewed by Urtiss (*12*). More recently the presence of complex ions in solutions containing copper(III), periodate or tellurate ions, and hydroxide ions has been recognized by the isolation of compounds (*8, 9*), by the elimination of the possibility of a peroxidic species (*7*), and by the magnetic susceptibility characteristic of the odd-electron copper (III) state (*9*). The copper(III) complex ions are stable in potassium and sodium hydroxide solutions, but unstable in ammonium, lithium and tetramethylammonium hydroxide solutions (*12*). Lister (*7*) reported that the use of stannate, stibnate, and selenate ions did not yield stable copper(III) complex ions. Beck (*3, 4*) found that titrations using copper(III) are best carried out in solutions containing potassium hydroxide.

The work which is described here includes the preparation of solutions, methods for detecting the end point in titrations, and studies of the oxidation of some selected compounds. Unless otherwise indicated, all calculations are based on the assumption that the copper(III)–copper(II) system is the only active redox couple.

PREPARATION OF COPPER(III) SOLUTIONS

Copper(II) has been oxidized to copper(III) in alkaline solution by electrolysis (*12*), with hypochlorite (*7*), and with potassium persulfate (*3, 4, 8, 9, 12*), the latter in the presence of periodate or tellurate ions. All solutions in this work were made by the persulfate method. Attempts to prepare stable copper(III) complexes with phosphate, perchlorate, arsenate, chromate, plumbate, molybdate, and tungstate ions were unsuccessful. In an attempt to reduce the cost of the periodate reagent, potassium iodate was used as a starting material in place of potassium periodate. The increased amount of potassium persulfate required was found to give an unstable solution which deposited potassium sulfate slowly for over 2 weeks in both the storage bottle and the buret. When the alkaline copper(III) solution is stored in glass bottles, the bottle is badly attacked, and, as the alkali is consumed, precipitates containing copper are formed. If the solution is stored in polyethylene bottles, no apparent decomposition occurs.

The following procedure was found to yield stable solutions containing about 0.05*M* diperiodato cuprate(III) or ditellurato cuprate(III) complexes. To 900 ml. of boiling distilled water in a 2-liter beaker equipped with a mechanical stirrer, add 12.5 grams of copper(II) sulfate pentahydrate. When dissolved, add 57.5 grams of potassium periodate or 44 grams of telluric acid ($H_2TeO_4 \cdot 2H_2O$). Carefully add a solution of 67.5 grams of potassium hydroxide dissolved in the smallest amount of water possible. At this point the mixture will be deep green (periodate) or will have a dark green precipitate (tellurate). Add a total of 60 grams of solid potassium persulfate in small portions at 1-minute intervals. Boil for 15 to 20 minutes to decompose the excess potassium persulfate, cool to room temperature, dilute to 1 liter, and store in a polyethylene bottle. The final solution is deep brown in color and deposits no precipitate on standing.

The brown solution on evaporation to dryness yields a brown residue which may be powdered and stored. Re-solution of the residue in distilled water gives a solution which has all the characteristics of the unevaporated solution.

END POINT DETECTION IN TITRATIONS

Because the copper(III) solutions are so intensely colored, a self-indicating reagent appeared to be available. Beck had reported this to be the case in microtitrations (*3, 4*), although he did state that in some titrations colored precipitates appeared. In titrations with 0.05*M* potassium diperiodato cuprate(III), blue or green solutions and greenish yellow precipitates were almost always found before the solution turned dirty brown, showing a large excess of reagent. A qualitative study was therefore made on the behavior of copper(II) periodate solutions. When potassium periodate is added to a solution of copper(II) sulfate, a yellow-green solid is formed. As a solution of potassium hydroxide is added to the solid, the solid slowly dissolves yielding a deep green solution, and as the amount of potassium hydroxide increases, the solution finally becomes dark blue, Addition of water to the dark blue solution reverses the effects showing that the hydroxide ion concentration is the determining factor. This is not surprising in view of the ionization constants for periodic acid (*5, 6*). In titrations with 0.05*M* potassium ditellurato cuprate(III) a greenish yellow precipitate is almost always present before an excess of reagent is present. Qualitatively, a greenish yellow precipitate is formed when a solution of telluric acid is added to a solution of copper(II) sulfate, and this precipitate is soluble only when the concentration of potassium hydroxide is several times greater than that normally present in a titration. Because intensely colored solutions and precipitates are found, the end point is very difficult to detect visually at ordinary concentrations in spite of the intensely colored reagent which otherwise should be self-indicating.

A series of titrations were then tried in an attempt to use a potentiometric end point. Since the solutions used contained 0.2 to 0.5*M* potassium hydroxide, the possible electrodes are limited, and measurements were finally made with platinum-calomel electrodes. In titrations of sodium tartrate and potassium ferrocyanide, the observed potential gradually increased up to the point where a visual excess of copper(III) reagent was present. No sharp increase was observed in the vicinity of the equivalence point. Therefore, attempts to use a potentiometric end point were abandoned.

Just beyond the equivalence point in titrations with copper(III) reagents both copper(II) and copper(III) would be present. Thus with both parts of an electrochemical couple present the dead-stop end point method should be applicable (*11*). A sharp end point was found with 100 to 200 mv. applied between platinum electrodes using both diperiodato cuprate(III) and ditellurato cuprate(III) for the oxidation of sodium thiosulfate in sodium bicarbonate solution and potassium cyanide, potassium ferrocyanide, and potassium arsenite in 0.5*M* potassium hydroxide. As a result, the dead-stop technique was used for all titrations of substances which had reasonable reaction rates in preliminary titrations.

[1] Present address, U. S. Army.

OXIDATIONS OF INORGANIC SUBSTANCES

The general procedure used in the investigation with inorganic substances was to pipet volumes of solutions of known concentration into beakers and to add concentrated potassium hydroxide solution until the mixture was 0.5*M* in potassium hydroxide. Preliminary titrations were made with copper(III) reagent solutions using visual end points in order to observe the formation of precipitates and to check the stoichiometry. For cases which appeared to be favorable, further titrations were made using the dead-stop end point method.

The oxidation of potassium iodide, potassium iodate, and sodium bisulfite was very slow and not suitable for volumetric use.

The oxidation of potassium ferrocyanide by diperiodato cuprate(III) was reasonably rapid. On visual observation duplicate samples required 33 and 35 ml. of reagent solution. The dead-stop method gave a sharp break at the end point, but the reproducibility of duplicate titrations was not improved. Calculation of the number of equivalents of oxidizing agent consumed per mole of ferrocyanide gave values in the range 4 to 4.5. These values do not correspond to any known stoichiometry. Because the precision was poor and the oxidation products were unknown, no further work was done with this system.

Since Beck had reported (*3*) the oxidation of arsenite to arsenate by diperiodato cuprate(III), this system was investigated further. Aliquots of arsenic(III) solution in 0.5*M* potassium hydroxide solution which should have consumed 20 ml. of diperiodato cuprate(III) solution were found to require only 3 to 8 ml. using the visual end point. In most cases blue or green precipitates or colored solutions interfered badly. The reverse titration—i.e., arsenic(III) added to copper(III)—did not improve the results. The use of the dead-stop method improved the results somewhat, 16.7 to 19 ml. being required compared to 25 ml. expected. Essentially the same results were found using ditellurato cuprate (III) solution with the exception that the precision was somewhat better. When some of the supernatant liquid from one of the diperiodato cuprate(III) oxidations was tested with nitric acid and silver nitrate solution, an appreciable amount of iodate was found. It must be concluded that not only the copper(III) but also the periodate is oxidizing the arsenic(III). Therefore quantitative results would not be expected.

Beck (*3*) reported that potassium cyanide was oxidized to potassium carbonate and nitrate by copper(III) reagents. With 0.05*M* diperiodato cuprate(III) and ditellurato cuprate(III) the oxidation of cyanide in 0.5*M* potassium hydroxide was moderately rapid, but the visual detection of the end point was difficult since precipitates and intense colors were present. The dead-stop end point gave a sharp break with either reagent. The ditellurato cuprate(III) reagent gave better reproducibility and was therefore used for the rest of the work. It was found that 50-ml. aliquots of 0.01*M* cyanide solution required 21.8 ± 0.2 ml. of approximately 0.05*M* copper(III) solution. These results suggest that two equivalents of oxidizing agent are required per mole of cyanide ion and that the product might be cyanate ion:

$$KCN + O \longrightarrow KCNO$$

Cyanate ion is known to hydrolyze in potassium hydroxide solution to yield ammonia and carbonate. The oxidation was therefore run in a Kirk microdiffusion cell with Nessler's reagent in the diffusion cup. A strong test for ammonia was found and the test carried out in the same way without cyanide ion was negative. The evidence found is all in agreement with the consumption of two equivalents of oxidizing agent per mole of cyanide ion.

Beck (*3*) also reported the oxidation of thiosulfate ion. In this work the oxidation of thiosulfate ion by both diperiodato cuprate (III) and ditellurato cuprate(III) was very slow in 0.5*M* potassium hydroxide. In saturated sodium bicarbonate solution, thiosulfate ion is oxidized erratically by the periodate reagent because periodate also is an oxidizing agent. In the same medium however, the tellurate reagent oxidizes thiosulfate ion with the consumption of approximately 1 equivalent per mole and the end point is best detected with the dead-stop method. The reproducibility of the titration was ± 0.1 ml. when 25 ml. were being used.

Table I. Oxidation of Potassium Cyanide and Sodium Thiosulfate by $K_9Cu(TeO_6)_2$

	KCN, *M*	$Na_2S_2O_3$, *M*	Cu(III), *N* Calcd.	Cu(III), *N* From KCN	Cu(III), *N* From $Na_2S_2O_3$
Trial 1	0.1221	0.1377	0.0489	0.0626 0.0619 0.0617	0.0484 0.0496 0.0482 0.0483
Trial 2	0.01221	0.01377	0.0435	0.0408 0.0408 0.0382	0.0357 0.0339

Since the preliminary experiments with potassium cyanide and sodium thiosulfate seemed promising, a cyclic experiment was designed to completely check the titrations of these two substances. A sample of copper(II) sulfate pentahydrate was analyzed iodometrically and found to be 99.8% pure on the basis of the hydrate. A solution of ditellurato cuprate(III) was prepared from a known weight of the copper(II) sulfate using an excess of oxidizing agent to be certain that all the copper was in the +3 state. A solution of potassium cyanide was standardized with silver nitrate, and a solution of sodium thiosulfate was standardized iodometrically against potassium iodate. Aliquots of the thiosulfate solution were titrated in saturated sodium bicarbonate solution to a dead-stop end point. Aliquots of the cyanide solution were titrated in 0.5*M* potassium hydroxide solution to a dead-stop end point. In all cases the total volume of the solution titrated was 100 ml. and the volume of copper(III) reagent varied from 15 to 40 ml. The normality (equal to the molarity) of the copper(III) solution was calculated from these titrations assuming 2 equivalents per mole for the cyanide and 1 equivalent per mole for the thiosulfate. The results are given in Table I. These results indicate that the concentration plays an important role, particularly with the cyanide where, at higher concentration, the oxididation is less than 2 equivalents per mole and at lower concentrations more than 2 equivalents per mole are required. It must be concluded that these oxidations are not satisfactory for general use.

OXIDATIONS OF ORGANIC SUBSTANCES

In an attempt to apply copper(III) oxidations to organic analysis, experiments were tried with cinnamic acid, malonic acid, acetone, and ethyl alcohol. In all cases the rate of oxidation by diperiodato cuprate(III) was too slow at room temperature to be of any value in volumetric analysis. At higher temperatures the rates were greater, but the volume of reagent solution consumed had no relation to the amount of compound added.

The oxidation of glucose has been extensively used by Beck (*2*, *4*) for the estimation of blood sugar levels. Applying the method to the titration of 0.01*M* glucose in 0.5*N* potassium hydroxide with 0.05*M* diperiodato cuprate(III) was found to be impractical. The rate of oxidation was very slow, highly colored solutions and precipitates were formed, and the process was not stoichiometric. If some of the supernatant solution which should contain only periodate was added to dilute nitric acid, a white precipitate was formed on the addition of silver nitrate showing the presence of iodate. Therefore, there are two competing oxidations, one by copper(III) and the other by periodate. No combination of temperature, alkalinity, and glucose concentration could be found which gave reproducible, stoichiometric results. The oxidation of glucose by ditellurato cuprate(III) gave the same limitations as found using the diperiodato cuprate (III). Tellurite could be detected in the presence of tellurate in 6*N* hydrochloric acid by reduction to tellurium metal with sulfurous acid formed on adding sodium bisulfite. The pre-

cipitate formed in oxidations of glucose with ditellurato cuprate (III) contained tellurite. Therefore, again competing oxidations were present, which indicated that further work with glucose was futile.

The oxidation of tartrate ion starting with disodium tartrate dihydrate by either diperiodato cuprate(III) or ditellurato cuprate(III) was found to be subject to the same limitations as the oxidation of glucose. The rate of oxidation was reasonable, but highly colored solutions and precipitates prevented the use of a visual end point. No break was found in the plot for the potentiometric titration and titrations with the dead-stop end point were not reproducible. In addition iodate was found in the mixture from diperiodato cuprate(III) oxidation.

In connection with the oxidation by the complexing anion, the 2 to 1 ratio found in the copper(III) complex is not necessarily found in the compound which can be isolated containing copper (II). For example, $Cu_5(IO_6)_2$ and $H_2Cu_4(IO_6)_2$ have been isolated (*10*). Hence periodate is available for oxidation as soon as any copper(III) is reduced if not before.

CONCLUSION

Applications of copper(III) oxidation at the millimole level are limited by the difficulty of end-point detection, the uncertainty of product composition, and the possible competition via the oxidizing action of the complexing anion. At the milligram level these difficulties may well be minimized so that empirical methods yield satisfactory results (*3*, *4*).

LITERATURE CITED

(1) Beck, G., *Anal. Chim. Acta*, **9**, 241–7 (1953).
(2) Beck, G., *Mikrochemie ver Mikrochem. Acta*, **35**, 169–73 (1950); **38**, 1–10 (1951); **39**, 22–9, 147–51 (1952); **40**, 258–64 (1953).
(3) *Ibid.*, **36**, 245–50 (1950).
(4) *Ibid.*, **38**, 152–9 (1951).
(5) Crouthamel, C. E., Hayes, A. M., and Martin, D. S., *J. Am. Chem. Soc.*, **73**, 82–7 (1951).
(6) Crouthamel, C. E., Meek, H. V., Martin, D. S., and Banks, C. V., *Ibid.*, **71**, 3031–5 (1949).
(7) Lister, M. W., *Can. J. Chem.*, **31**, 638–52 (1953).
(8) Malaprade, L., *Compt. rend.*, **204**, 979–80 (1937).
(9) Malatesta, L., *Gazz. chim. ital.*, **71**, 467–74, 580 (1941).
(10) Smith, G. F., "Periodic Acid and Iodic Acid," **5th** ed., p. 5, G. F. Smith Chemical Co., Columbus, Ohio, 1950.
(11) Stone, K. G., and Scholten, H. G., ANAL. CHEM., **24**, 671–4 (1952).
(12) Urtiss, M., *Rec. trav. chim.*, **44**, 425–34 (1925).

RECEIVED for review September 9, 1954. Accepted November 16, 1954. Presented before the Division of Analytical Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y. Abstracted in part from the M.S. Thesis submitted by Donald A. Keyworth, June 1954.

Determination of Zirconium in Magnesium Alloys Using *p*-Bromo- or *p*-Chloromandelic Acid

ROLAND A. PAPUCCI, *F. C. Broeman and Co., Cincinnati 10, Ohio,* and
JOSEPH J. KLINGENBERG, *Xavier University, Cincinnati 7, Ohio*

Successful application of *p*-bromo- and *p*-chloromandelic acids to the determination of zirconium in steel and aluminum alloys suggests a similar application to zirconium-containing magnesium alloys. Using these reagents, a rapid and reliable procedure was developed which can be applied to all types of magnesium alloys.

WARTIME conditions and the constant search for high temperature alloys for use in the turbojet industry have led to a more complete study of magnesium base alloys. The addition of small amounts of zirconium to magnesium and magnesium alloys was found to improve the operating temperatures and the grain structure without affecting the creep resistance or machinability.

To meet this and other new metallurgical advances, accurate and more rapid methods for the determination of zirconium in small concentrations are needed. The phosphate method (*1*) is subject to error in the low concentration range and requires excessive time especially when the zirconium content is lower than 0.25%. The feasibility of using *p*-bromo- or *p*-chloromandelic acid for the determination of zirconium in steels and in aluminum alloys has been demonstrated (*2*, *3*). The development of a method utilizing these reagents which would combine speed with accuracy and which could be applied to all types of magnesium alloys was undertaken. Such a method might also compare favorably with the alizarin red S colorimetric method (*4*) which is also available for the determination of zirconium in magnesium.

Zirconium occurs in commercial magnesium alloys in acid-soluble and acid-insoluble forms. The strength characteristics of the specific alloys are related to the soluble zirconium content of the alloy. The acid-insoluble zirconium is usually small in comparison to the acid-soluble content. In some cases the determination of the total zirconium content is desired; in others both the amount of acid-soluble and acid-insoluble zirconium. Consequently two procedures were developed. One procedure describes the determination of acid-soluble zirconium only. The other describes the determination of the soluble and insoluble forms. Both are applicable to all types of magnesium alloys.

PROCEDURE

Determination of Soluble Zirconium. A sample of 0.25 to 2.0 grams (amount depending on the zirconium content) is placed in a 250-ml. beaker. An amount of hydrochloric acid (1 to 4) corresponding to 80 ml. per gram of alloy dissolved is added. The beaker is covered with a watch glass and warmed. When the reaction is complete, the contents of the beaker are cooled to room temperature and the watch glass is rinsed with small amounts of water. The solution should be clear. If a residue can

Table I. Determination of Acid-Soluble Zirconium in Magnesium Alloys

Sample	Zirconium Present, %	Zirconium Found, %			
		Phosphate	Alizarin red S	*p*-Chloromandelic acid	*p*-Bromomandelic acid
1	0.44	0.42	0.44	0.44 0.44	0.45 0.45
2	0.46	0.45	0.45 0.44	0.47 0.47 0.47	0.46
3	0.48	0.46 0.47	0.47	0.48 0.48 0.47 0.48	0.49 0.48
4	0.95	0.95 0.95	0.97 0.97	0.98 0.97 0.98 0.98 0.98	0.98 0.98 0.98

Table II. Determination of Acid-Soluble and Acid-Insoluble Zirconium in Magnesium Alloys

			Zirconium Found, %							
			Phosphate		Alizarin red S		*p*-Chloromandelic acid		*p*-Bromomandelic acid	
Sample[a]	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.
Dow 72480	0.57	0.02	0.56	0.01	0.56	0.02	0.58	0.03	0.58	0.03
			0.55	...	0.56	..	0.58	...	0.59	0.03
									0.58	0.03
									0.58	0.03
Dow 72992	1.10	55.10	1.09	54.89	1.12	..	1.14	55.22	..	..
					1.14	..	1.14	55.27	..	..
					1.12	..	1.14	55.20	..	..
Dow 72993-1	0.20	0.04	0.20	0.03	0.21	0.04	0.21	0.04	0.21	0.04
			0.20	...	0.20	..	0.21	0.04	0.21	0.04
									0.21	0.04
Dow 72993-2	0.62	0.06	0.61	0.06	0.62	0.05	0.63	0.06	0.63	0.07
					0.61	0.05	0.63	0.06		
							0.62	0.06		
							0.63	0.06		
							0.63	0.06		

[a] Samples and analyses, courtesy of Dow Chemical Co., Midland, Mich.

Table III. Determination of Total Zirconium in Magnesium Alloys Containing Acid-Soluble and Acid-Insoluble Zirconium

	Zirconium Found, %		
Sample	Phosphate	*p*-Chloromandelic acid	*p*-Bromomandelic acid
5	0.47	0.49	0.49
		0.48	0.49
		0.48	0.49
			0.49
6	0 92	0.92	0.93
	0.92	0.92	
		0.93	
		0.93	
7	0.89	0.92	
		0.92	
		0.91	
		0.92	
8	0.70	0.73	0.73
		0.73	
		0.73	
9	0.51	0.53	
	0.51	0.53	
		0.53	
		0.54	

be seen, acid-insoluble zirconium is present. Very small amounts of acid-insoluble zirconium (less than 0.04%) may not be visible to the naked eye. If such a residue is present or suspected, it is removed by filtration before proceeding.

A solution of 50 ml. of 0.1*M* *p*-bromo- or *p*-chloromandelic acid per 0.25 gram of sample is then added with constant stirring. The corresponding amount of pure solid reagent may also be added directly to the solution. The contents are stirred, digested at about 80° to 85° C. for 20 minutes, cooled, and filtered through Whatman No. 40 paper. The precipitate is washed 10 to 12 times with water, charred slowly in a weighed platinum crucible, and ignited at 1000° C. The difference in weight represents zirconium oxide. The residue can also be weighed directly in a tared weighing dish after being brushed from the platinum crucible.

$$\% \text{ zirconium} = \frac{\text{weight } ZrO_2 \times 0.7403 \times 100}{\text{weight of sample}}$$

Determination of Soluble and Insoluble Zirconium. A 0.25- to 2.0-gram sample is placed in a 250-ml. beaker, the amount used depending on the approximate zirconium content. An amount of hydrochloric acid (1 to 4) corresponding to 80 ml. per gram of alloy dissolved is added slowly until the vigorous reaction has ceased. Sulfuric acid (1 to 4) may be used in place of the hydrochloric acid. The contents are warmed to ensure complete reaction. Insoluble zirconium, not actually alloyed with the magnesium, will be evident at this stage appearing as minute dark particles. Very small amounts (less than 0.04%) may not be visible. The suspension is cooled and filtered on Whatman No. 40 paper. The soluble zirconium in the filtrate can be determined by the described procedure.

The insoluble matter and filter paper are transferred to a platinum dish, charred slowly, and ignited at 1000° C. The residue is fused with 1 to 3 grams of potassium acid sulfate, dissolved in hydrochloric or sulfuric acid (1 to 1) and, if only insoluble-zirconium is to be determined, the volume adjusted to about 50 ml. If the total zirconium content is to be determined, the solution is combined with the original filtrate. Fifty milliliters of 0.1*M* *p*-bromo- or *p*-chloromandelic acid per 0.25-gram sample are added (more if the zirconium content is high in the sample). The contents are digested on a hot plate at about 80° to 85° C. for at least 20 minutes, cooled to room temperature, and filtered on Whatman No. 40 paper, using some ashless pulp. The precipitate is washed at least 12 times with small portions of warm water, the residue charred in a weighed platinum dish, and finally ignited at 1000° C. The residue can also be weighed directly in a tared weighing dish after being transferred from the platinum crucible. Per cent zirconium is calculated according to equation.

RESULTS

Tables I, II, III, and IV give the results obtained for zirconium in acid-soluble and acid-insoluble forms in various kinds of magnesium alloys using the halomandelate reagents. Comparative results by the alizarin red S and the phosphate method are also included. Transmittance measurements for the alizarin red S method were made with a Model DU Beckman spectrophotometer modified to hold two 100-mm. silica window–Vycor body cells at a wave length of 510 mμ.

Table IV. Complete Analysis of Magnesium Alloys Containing Acid-Soluble and Acid-Insoluble Zirconium

Zirconium calculated as total zirconium

		Zirconium Found, %			
Compn. Alloy, %		Phosphate	Alizarin red S	*p*-Chloromandelic acid	*p*-Bromomandelic acid
Th	2.98	0.70	0.72	..	0.73
Zn	2.25	0.70	0.71	..	0.73
R.E.	0.07	0.71	..	..	0.73
Mn	0.06				0.73
Cu	0.02				0.73
Si	0.008				
Ni	0.005				
Mg	Balance				
Zn	4.06	0.54	0.55	0.56	0.57
Mn	0.12	0.54	0.55	0.56	
Cu	0.02	0.55	0.54	0.57	
Si	0.008			0.56	
Fe	0.005			0.56	
Ni	0.001			0.56	
Mg	Balance				
Ce	2.89	0.81	0.82	0.83	0.83
R.E.	3.65	0.81	0.82	0.83	0.83
Zn	2.60			0.82	0.83
Mn	0.10			0.83	0.84
Cu	0.03			0.83	
Si	0.006			0.83	
Fe	0.005				
Ni	0.001				
Mg	Balance				

The halomandelate method was considerably more rapid than the phosphate method and equal to if not better than the colorimetric method. The authors prefer the halomandelate procedure.

LITERATURE CITED

(1) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," pp. 446–51, Wiley, New York, 1929.
(2) Klingenberg, J. J., and Papucci, R. A., ANAL. CHEM., 24, 1861 (1952).
(3) Papucci, R. A., Fleishman, D. M., and Klingenberg, J. J., *Ibid.*, 25, 1758 (1953).
(4) Wengert, G. B., *Ibid.*, 24, 1449 (1952).

RECEIVED for review September 3, 1954. Accepted November 16, 1954.

Ammonia Determination and Sample Preparation for Mass Spectrometer by a Micro Diffusion Method

S. W. MAYER, F. H. KELLY, and M. E. MORTON

Radioisotope Unit, Veterans Administration Hospital, Long Beach, and School of Medicine, University of California, Los Angeles, Calif.

In order to analyze rapidly large numbers of samples of nitrogenous metabolic products and chromatographic fractions, a simplified, rapid diffusion method has been developed and extended to a range from 0.5 γ to 10 mg. of nitrogen. The standard deviation in ammonia recovery determinations was 2% for quantities of 10 γ or larger, and 0.2 γ for quantities less than 10 γ. The method is being used to prepare samples for mass spectrometer determinations, supplanting the Kjeldahl distillation method.

IN THE course of an investigation of nitrogen metabolism with nitrogen-15, it became necessary to develop a method for the determination of microgram quantities of nitrogen in several organic and inorganic materials. Inasmuch as such determinations of nitrogen frequently are resolved into determinations of ammonia, Conway (*1*) and Kirk (*3*) have devised ammonia diffusion cell methods involving titrimetry. Linderstrom-Lang and Holter (*4*) and Seligson and Seligson (*5*) employed single cell microdiffusion arrangements in which the ammonia was absorbed in an acid solution suspended above the alkaline sample. The authors have modified the latter method to extend its range from 0.5 γ to 10 mg. of nitrogen, and have employed the procedure as a time-saving means for preparing ammonia samples prior to isotopic analysis in the mass spectrometer. The method is rapid, utilizes readily available materials, and is so simple manipulatively that it is well adapted to the routine handling of large numbers of samples by technicians.

PROCEDURE

Diffusion of the ammonia is carried out in a 30-ml. bottle (30 mm. in diameter, 70 mm. high). A one-hole rubber stopper (No. 0) which holds a glass rod, seals the bottle. The base of the glass rod is 20 mm. from the bottom of the vessel and is slightly broadened so that the rod holds ten borosilicate glass "helices" which had been placed on the rod before the base of the rod had been fused and broadened. The helices serve to hold a relatively large quantity of sulfuric acid solution on the rod. For samples liberating milligram quantities of ammonia the rod is loaded with 50% sulfuric acid; 5% sulfuric acid is adequate for the microgram range.

The sample is placed in the bottle; eight glass beads are added to facilitate mixing and diffusion, and enough distilled water is pipetted in to bring the liquid volume to 3.00 ml. Then 1.5 ml. of saturated potassium carbonate are added, and the bottle is immediately sealed with the stopper holding the glass rod. In order to expedite diffusion, the bottle is rotated for 30 minutes (with its axis horizontal) at 45 r.p.m., while held in a clamp so that it is 13 cm. from the center of rotation. During the rotation two stationary infrared lamps heat the bottle to a temperature of 65° ± 2° C.

The Bock-Benedict modification (*2*) of Nessler's reagent is used for the ammonia determination, with the Beckman DU spectrophotometer at the 418 mμ peak. For microgram quantities of ammonia, 3.00 ml. of the 1 to 10 (volume per volume of water) Nessler's solution are transferred to a 1-cm. Corex absorption cell. The stopper is then removed from the 30-ml. diffusion bottle, and the base of the glass rod is put directly into the Nessler's solution in the Corex cell. The rod is moved vigorously up and down several times to secure efficient rinsing and mixing action, 5 minutes are allowed for color development, and the absorbance of the solution is compared to that formed with a suitable blank.

For milligram quantities of ammonia, the 50% sulfuric acid solution which has absorbed the ammonia is washed from the rod into a volumetric flask. Suitable aliquots are then diluted and added to Nessler's reagent so that the reagent is finally diluted to 1 to 10. Then the increase in absorbance produced by the ammonia is measured spectrophotometrically.

Ordinarily, a dozen diffusion bottles are rotated simultaneously for 30 minutes. The sulfuric acid (containing the diffused nitrogen-14 and nitrogen-15 as NH_4^+) from each of those bottles holding samples for mass spectrometer analysis, is transferred to the two-legged flask (*6*) used for reaction of ammonia with hypobromite to furnish the nitrogen gas at the manifold of the mass spectrometer.

RESULTS

The results of a study of the effect of time of rotation on the recovery of ammonia are summarized in Figure 1. Complete recovery was attained in 20 minutes for 1-γ and 1-mg. samples of ammonia nitrogen. The solid circles in Figure 1 summarize the experimental data for the recovery of 1 mg. of ammonia nitrogen when the rotation was carried out at 25° C., rather than at the regular temperature of 65° C. At room temperature, at least 40 minutes were required to obtain complete recovery.

A series of measurements of the recovery of ammonia nitrogen in the range from 1 to 15 γ provided a linear calibration curve.

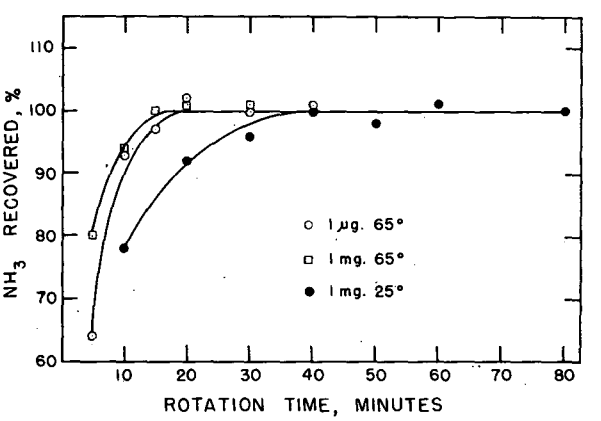


Figure 1. Effect of time of rotation on recovery of ammonia

Several sets of twelve replicate determinations at a single concentration were made in order to evaluate the reproducibility of the standard procedure. The mean recoveries of ammonia nitrogen and the corresponding standard deviations for 1.00 γ, 10.0 γ, and 1.00 mg. were, respectively, 1.04 ± 0.18 γ, 9.91 ± 0.21γ and 0.999 ± 0.19 mg. Recoveries of 10.0 γ of ammonia nitrogen added to blood or urine of known ammonia content were 10.1 ± 0.22 γ and 10.0 ± 0.22 γ, respectively.

DISCUSSION

Kirk (*3*) has pointed out that the alkaline solutions in ammonia diffusion methods should have shallow liquid layers. Accordingly, in the present procedure the horizontal position of the bottle, the use of glass beads, and the rotation of the bottle were designed to facilitate diffusion. The use of heat lamps proved to be convenient for raising the temperature and increasing the rate of

processes involved in this method. When such heating would lead to significant hydrolysis of amides present, the diffusion should be carried out at lower temperatures for correspondingly longer time intervals.

The accuracy of the Nessler spectrophotometric method appears to be somewhat inferior to the within 1% accuracy observed in Conway's and Kirk's titrimetric procedures. The ease of manipulation in the present procedure, in which the color is developed directly in the absorption cell, is advantageous, however, when large numbers of microanalyses are to be run in a relatively routine way. The analyses of the large numbers of nitrogenous materials separated, for example, from chromatographic columns or from the products of tissue slice or homogenate metabolism could be facilitated by this simple procedure. The ammonia can be stoichiometrically produced (*3*) from urea, amides, amines, total nitrogen, and nonprotein nitrogen.

The microdiffusion technique has proved to be highly satisfactory and convenient for preparing nitrogen samples for isotope ratio determination with the mass spectrometer. The ammonia sample is obtained directly in an interference-free form for the usual treatment with hypobromite (*6*) to produce nitrogen gas.

LITERATURE CITED

(1) Conway, E. J., and Byrne, A., *Biochem. J.*, **27**, 419 (1933).
(2) Hawk, P. B., Oser, B. L., and Summerson, W. H., "Practical Physiological Chemistry," 12th ed., p. 1230, Blakiston, Philadelphia, 1947.
(3) Kirk, P. L., "Quantitative Ultramicroanalysis," Chap. 7, Wiley, New York, 1950.
(4) Linderstrom-Lang, L., and Holter, H., *Z. physiol. Chem.*, **220**, 5 (1933).
(5) Seligson, D., and Seligson, H., *J. Lab. Clin. Med.*, **38**, 324 (1951).
(6) Sprinson, D. B., and Rittenberg, D., *Naval Medical Bull.*, Supplement on Preparation and Measurement of Isotopes, p. 82, 1948.

RECEIVED for review July 15, 1954. Accepted November 12, 1954.

Procedure for Determination of Diffusion Coefficients of Gases and Nongaseous Solutes for Membranes

SVEND G. JOHNSEN and JOHN ESBEN KIRK

Division of Gerontology, Washington University School of Medicine, St. Louis, Mo.

A procedure was developed for determination of the diffusion rates of gaseous and nongaseous solutes from one fluid phase to another through biological or inanimate membranes. The procedure permits determination under a constant total pressure of the diffusion coefficients for several solutes within a single experiment.

PROCEDURES for determination of diffusion rates of gases through membranes have been described by Krogh (*3*) and Wright (*5*). The apparatus devised by Krogh permits measurement of diffusion rates of oxygen and carbon dioxide in separate experiments, whereas the apparatus used by Wright is applicable only to the study of the diffusion of carbon dioxide. The procedure described in the present publication represents a further development of Krogh's diffusion method. It permits measurement of the diffusion rates of gases and nongaseous solutes from one liquid phase to another through biological or inanimate membranes, utilizing the principle for sample transfer and gas analysis employed by Kirk and Hansen (*2*).

PROCEDURE

Apparatus. DIFFUSION APPARATUS. The details of the assembled diffusion apparatus are shown in Figure 1. The apparatus consists of two 50-ml. glass syringes (A. S. Aloe precision syringes); the bottoms of the barrels have been removed and the barrel tops have been ground plane. The membrane is interposed between the ground barrel tops of the syringes and is held in place by two metal diaphragms with circular openings and two hard rubber 1.25-inch slip joint washers. A cannula of stainless steel about 1 mm. in outside diameter (hypodermic needle, gage 19) is inserted through each rubber ring. The tips of the needles are cut off at a right angle and do not extend beyond the inner surface of the rings. Airtightness of the perforation canal is ensured by sealing the place of entrance of the needle with cement (Sealit, Fisher Scientific Co.). The outer ends of the needles are closed by insertion of a one-way metal stopcock with two male outlets. Rotation of the diffusion apparatus is provided by an electric motor, as shown in the insert of Figure 1. A screw-shaped glass piece about 1 cm. in length, placed in each compartment, effects stirring of the solution during the rotation of the apparatus.

The metal diaphragms used in the diffusion apparatus are made of chromium-plated steel. Sets with beveled circular openings 25, 20, 15, and 10 mm. in diameter have been found suitable.

The rubber washers should be tested for gas permeability. With the hard-rubber washers used by the authors no measurable loss of gas was observed over a 2-hour period in experiments in which the compartments were filled with carbon dioxide–aerated water and a steel plate interposed between the rubber rings.

EQUIPMENT FOR GAS ANALYSIS. The equipment for gas analysis includes one, or preferably two, Van Slyke manometric apparatus with extraction chambers provided with calibration marks at 0.5-, 2.0-, 10.0-, and 50.0-ml. volumes. A stainless steel cannula 75 mm. in length and about 2 mm. in outside diameter (hypodermic needle, gage 15) with attached rubber tip is used for transfer of samples from the diffusion apparatus to the extraction chamber of the Van Slyke apparatus (*2*).

Reagents. The reagents for gasometric determination of carbon dioxide, oxygen, and nitrogen are described by Kirk and Hansen (*2*).

Technique for Use of Diffusion Apparatus. In diffusion studies on animal membranes the experiments are preferably carried out under sterile conditions. After the membrane, the metal diaphragms, and the rubber washers have been inserted between the syringe barrels, the apparatus is screwed firmly together. The metal stopcocks and the outside parts of the needles are secured in a fixed position by means of thin copper wire.

For experiments on gas diffusion about 50 ml. of buffer medium are heated in a beaker to 43° C., and the solution is aerated with the appropriate gas as described by Kirk and Hansen (*2*). Thirty to 40 ml. of the solution are then poured into one of the compartments of the apparatus, the screw-shaped glass piece is placed in position, and the plunger is inserted. Any free gas present is ejected, and the volume of solution introduced is determined by weighing. Buffer medium for the other compartment is then prepared and similarly introduced. The plungers require no special fastening, but will stay in place during the rotation of the apparatus. The diffusion apparatus is finally placed horizontally in the belts of the rotator (see insert, Figure 1) in a thermostat at 37° C., and rotation is started. It is convenient to place a shield of paper board or a rubber ring around each syringe barrel to keep the belts in place during the rotation. An elapsed time of 20 to 30 minutes is allowed to establish temperature equilibrium and initial penetration of the gas through the membrane.

The apparatus permits determination of the diffusion coefficients of several gases in the same experiment. Buffer medium aerated with oxygen may be used in one of the compartments,

and medium aerated with nitrogen or a nitrogen–carbon dioxide mixture in the other compartment. If the diffusion rate of carbon dioxide alone is studied, buffer medium aerated with this gas is employed on one side and unaerated medium on the other side.

For experiments with nongaseous solutes a mixture of equal volumes of buffer medium and an isotonic solution of the compound to be studied is introduced in one of the compartments, and buffer medium is employed on the other side.

At the end of the diffusion experiment the volume of solution remaining in each compartment is measured; this control ensures that holes in the membrane will not be overlooked. After the apparatus is disassembled, the membrane is removed. The part inside the openings of the diaphragms is carefully cut out and its area and weight are determined.

Analytical Technique for Gas Determinations. The transfer of samples from the diffusion apparatus to the extraction chamber of the Van Slyke apparatus is carried out as described for Procedure B by Kirk and Hansen (*2*). About 1 ml. of solution is ejected from the compartment of the diffusion apparatus before the introduction of the sample into the chamber. The exact measurement of the sample and the gasometric analysis are performed as described previously (*2*). In experiments in which the diffusion of oxygen and nitrogen are studied a 9-ml. sample is used for maximum accuracy. When carbon dioxide alone is studied samples of 1 to 2 ml. may be employed without loss of accuracy.

Interval between Withdrawal of Samples. If conditions of analysis permit, samples should be withdrawn in immediate succession from the two compartments of the apparatus at the beginning and end of a diffusion period. This will be possible in the case of diffusion studies on most nongaseous solutes and in investigations on gas diffusion when two Van Slyke apparatus are available. If only one Van Slyke apparatus is available, an interval corresponding to the time required for performing a gas analysis must elapse between the withdrawal of samples from the two compartments of the diffusion apparatus. This period is about 15 to 18 minutes in the case of oxygen and nitrogen determinations, and 5 to 6 minutes for carbon dioxide and bicarbonate determinations.

The appropriate length of each diffusion period depends on the area and thickness of the membrane, the diffusion coefficient of the compound, and the sensitivity of the analytical method. In gas diffusion studies on animal membranes 3 to 4 sq. cm. in area and 0.04 to 0.08 cm. in thickness, a 1- to 2-hour interval has been found suitable.

CALCULATION OF DIFFUSION COEFFICIENT

The diffusion coefficient is defined as the number of units of the substance diffusing through 1 sq. cm. of the membrane in 1 minute at a concentration gradient of 1 unit per ml. per cm. (*1*).

For experiments in which samples are withdrawn in immediate succession from the two compartments at the beginning and end of a diffusion period the coefficient may be calculated from the equation given by Pletscher and coworkers (*4*):

$$(c_3 - c_4) = (c_1 - c_2)e^{-k\frac{A}{L}\left(\frac{1}{V_1}+\frac{1}{V_2}\right)t} \qquad (1)$$

where

k = diffusion coefficient
c_1 = concentration of solute on donor side at beginning of period
c_2 = concentration of solute on recipient side at beginning of period
c_3 = concentration of solute on donor side at end of period
c_4 = concentration of solute on recipient side at end of period
A = area of membrane in square centimeters
L = thickness of membrane in centimeters
V_1 and V_2 = volumes of solution in the two compartments expressed in milliliters
t = time in minutes

In diffusion studies in which an interval elapses between the withdrawal of samples from the two compartments of the apparatus another formula for calculation of the diffusion coefficient must be employed:

$$k = \frac{q \times L}{A \times d \times t} \qquad (2)$$

where q represents the decrease in quantity of the compound

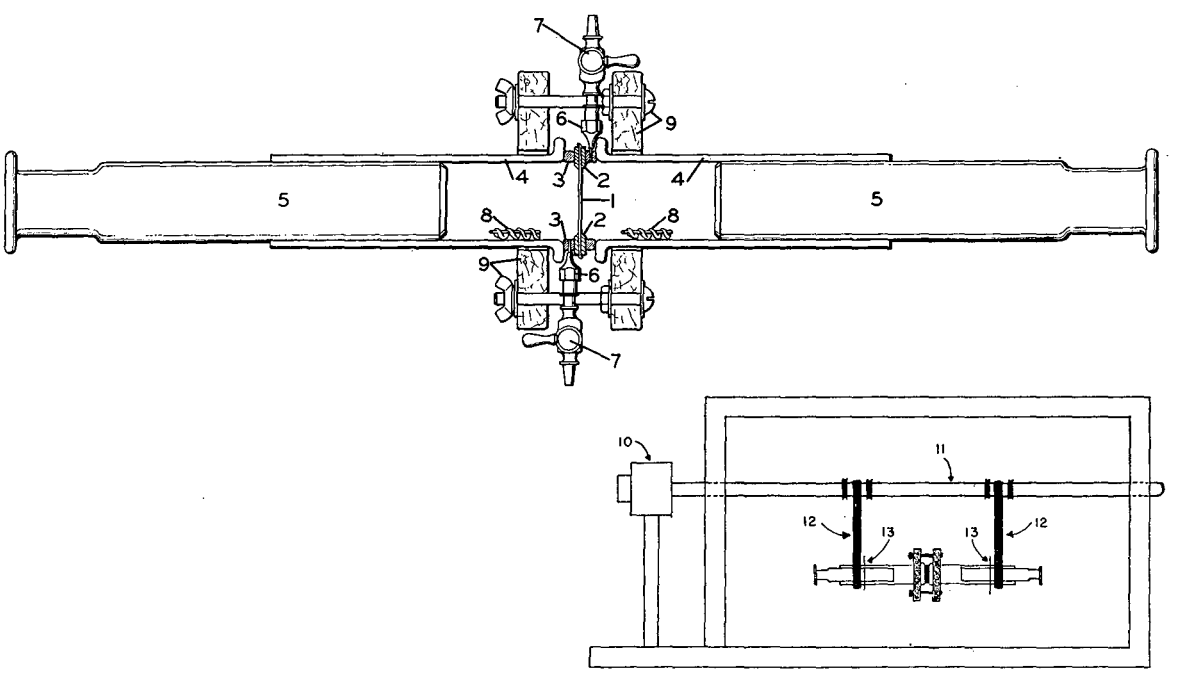


Figure 1. Schematic illustration of assembled diffusion apparatus

1. Membrane
2. Metal diaphragm with beveled circular opening
3. Hard rubber 1.25-inch slip joint washer
4. Syringe barrel
5. Syringe plunger
6. Stainless steel cannula (hypodermic needle, gage 19) with tip cut off at right angle
7. One-way metal stopcock with two male outlets
8. Screw-shaped glass piece to provide stirring of solution
9. Wooden clamp with brass screws
10. Motor
11. Metal axis
12. Belt
13. Paper board shield

(expressed in units) on the donor side or the increase in quantity on the recipient side, and d is the mean concentration difference (expressed in units per milliliter) during the diffusion period. The value of d is obtained by plotting the observed concentrations on the donor and recipient sides on graph paper in relation to time. Smooth curves are then drawn to connect the values on each side, and the mean concentration difference is calculated from the area limited by the curves.

Correction for Tissue Respiration. In diffusion studies on living membranes some oxygen will be used by the respiration of the tissue. If the experiment is carried out by withdrawal of samples in immediate succession from the two compartments, no correction for the tissue respiration is necessary if the volumes of fluid on the two sides are the same, the supposition being made that an equal amount of oxygen is consumed on both sides of the membrane.

In experiments in which a time interval elapses between the withdrawal of samples from the two compartments, the value of q in Formula 2 must be corrected for the amount of oxygen consumed by the tissue. If it is assumed that the oxygen used for the tissue respiration on the average has diffused through half of the membrane, the correction to be applied is equal to one half of the difference between the decrease in quantity of oxygen on the donor side and the increase in oxygen on the recipient side.

If a solution aerated with pure carbon dioxide is used in the donor compartment of the apparatus, the respiratory carbon dioxide can be disregarded.

DISCUSSION

The main advantages of the present procedure as compared with previous methods are the following: It permits determination of the diffusion coefficients of several solutes in the same experiment; it provides for the withdrawal of samples for analysis at any time during an experiment, thus making it possible to carry out successive diffusion periods within a single experiment; it permits an accounting for the diffusing quantities of a compound by comparison of the amounts disappearing on one side of the membrane with those appearing on the other side; and it involves the use of easily movable syringe plungers, thus ensuring against differences in total pressure on the two sides of the membrane.

LITERATURE CITED

(1) Hill, A. V., *Proc. Roy. Soc. London*, 104B, 39 (1928–29).
(2) Kirk, J. E., and Hansen, P. F., *J. Biol. Chem.*, **199**, 675 (1952).
(3) Krogh, A., *J. Physiol.*, 52, 391 (1918–19).
(4) Pletscher, A., Staub, H., Hunzinger, W., and Hess, W., *Helv. Physiol. et Pharmacol. Acta*, 8, 306 (1950).
(5) Wright, C. I., *J. Gen. Physiol.*, 17, 657 (1933–34).

RECEIVED for review September 7, 1954. Accepted December 13, 1954. Investigation supported in part by a research grant (PHS-891) from the National Heart Institute of the National Institutes of Health, Public Health Service. A demonstration of the technique was given at the 43rd meeting of the American Society of Biological Chemists, April 14 to 18, 1952, New York, N. Y.

Effect of Particle Size on the Characteristics of Silicic Acid Chromatographic Adsorbent

EARL W. MALMBERG

McPherson Chemical Laboratory, The Ohio State University, Columbus, Ohio

The effect of particle size on the efficacy of silicic acid in affording chromatographic separations has been studied with the purpose of obtaining the best adsorbent from commercially available materials. Procedures of sieving and ball-milling have been used to provide samples whose effectiveness was tested by their ability to separate the 2,4-dinitrophenylhydrazones of acetaldehyde and formaldehyde and the dinitrophenylosazones of glyoxal and methyl glyoxal. A procedure for obtaining a satisfactory adsorbent from a commercially available silicic acid is described.

THE influence of physical characteristics on the behavior of chromatographic adsorbents is generally ignored until difficulties arise which necessitate bringing certain variables under control. The influence of particle size on the chromatographic behavior of silicic acid is the subject of the present investigation. For some years commercially available silicic acid has been used as a chromatographic adsorbent and has given uniform characteristics except for certain variations in adsorptive strength which are to be expected. Recently the samples of silicic acid from the usual source have been completely different in particle size and apparently in their adsorption characteristics in general. Studies of particle size distribution, grinding, and chromatographic separations were made on various samples of silicic acid. A procedure for preparation of a very satisfactory adsorbent from a commercially available Mallinckrodt silicic acid is described as devised from these investigations.

GENERAL EXPERIMENTAL PROCEDURES

Throughout this work two rather sensitive chromatographic problems, the separation of the 2,4-dinitrophenylhydrazones of formaldehyde and acetaldehyde and of the 2,4-dinitrophenylosazones of glyoxal and methylglyoxal, were used as criteria of the chromatographic power of an adsorbent. These tests are referred to below simply as the formaldehyde-acetaldehyde and glyoxal-methylglyoxal separations, respectively.

The procedure (*1*) for the formaldehyde-acetaldehyde separation requires a prewash of V_{150} ml. (*2*) of 10% of acetone in ligroin (Skellysolve B), 0.5 mg. each of hydrazones in 5 ml. of 1:4 chloroform-ligroin (on a column of 14-mm. inside diameter), and development with 10% of ether in ligroin. Some of the best adsorbents did not require the prewash, but even with the best the separations were improved. The osazones, 0.1 mg. of each in 1 to 14 nitrobenzene-benzene, were placed on a column wet with benzene and developed with a solution of 5% of ether in 1 to 1 benzene-ligroin. This system has a complicating feature: Some adsorbents will not afford a satisfactory separation under any conditions, while other samples will give a good separation at slow flow rates but not at fast. From the profiles of the zones, this rate effect seems to be a result of a slow rate of desorption for some of the adsorption sites.

The ball-milling of the adsorbent was done in a Paul O. Abbè mill of 6-gallon size, 55 r.p.m. The mill was charged with 0.5 kg. of silicic acid and 1.5 kg. of 1-inch porcelain balls. Combinations of larger amounts of material and balls were found to be ineffective. In all cases the adsorbent was heated 4 hours at 200° C. before testing.

Each column was packed with the tube in position on a suction flask, the adsorbent being poured in with a stopcock on a safety bottle open to the atmosphere; after initial settling was completed, the stopcock was closed, and as the vacuum was established, the column further contracted. To prevent surface spreading, the upper surface of the adsorbent must be pressed

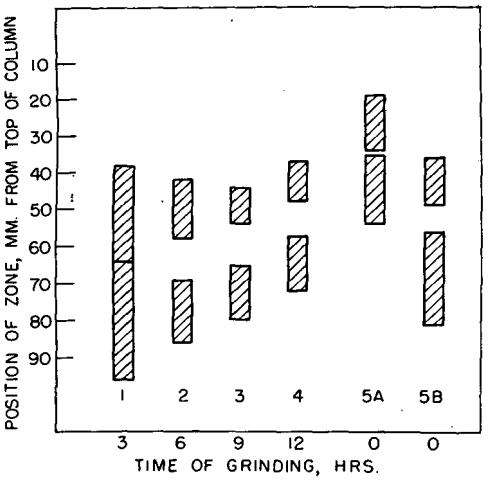


Figure 1. Zone positions with variations in particle size of adsorbent

Test of Mallinckrodt Ramsay and Patterson silicic acid with separation of dinitrophenylhydrazones of formaldehyde and acetaldehyde. Original material and samples ball-milled and mixed with Celite
1 to 4. Silicic acid mixed with 0.5 part of Celite 535
5*A* to 5*B*. Silicic acid as received; no Celite
1 to 5*A*. Identical procedure of development
5*B*. Additional development to improve comparison

Table I. Particle Size Distribution of Old Silicic Acid and New Coarse Adsorbent

(Percentage particle size classified with U. S. standard sieves)

Mesh Size	Sample I[a]	Sample II[b]
+80	2	52
−80, +150	5	12
−150, +200	12	8
−200, +300	73	16
−300	8	12

[a] Sample I, old silicic acid; satisfactory chromatographic properties.
[b] Sample II, new coarse silicic acid; unsatisfactory chromatographic properties.

down firmly with a dowel. All work was done with a so-called No. 1.5 column, of 14-mm. inside diameter.

A flow rate of 3 minutes for V_{150} ml. of ligroin for a 150-mm. column is regarded as fast, 6 to 8 minutes is satisfactory, and 10 to 12 minutes is slow. These rates are with the column completely wet. Positions of zones were measured on extruded columns, so that the results are completely free of any ambiguity which could result from distortions.

INVESTIGATION OF COARSE SILICIC ACID

A comparison of the particle size distribution of the older satisfactory adsorbent and the newer coarse material is shown in Table I.

Chromatographic characteristics of individual particle size fractions of sample II were studied with the following results. The −200, +300 fraction mixed with 0.5 part of Celite gave a satisfactory flow rate but no separation of the glyoxal-methylglyoxal system. When an amount of the −300 fraction equal to the amount of −200, +300 fraction was mixed in, the filtration was very slow but a satisfactory glyoxal-methylglyoxal separation was obtained. When the +80 fraction was ball-milled and mixed with Celite to give a satisfactory flow rate, a satisfactory formaldehyde-acetaldehyde separation was obtained but the glyoxal-methylglyoxal system was spread very badly.

When the original material of sample II was subjected to various lengths of time of ball-milling and the products were mixed with Celite, usually one of the two test separations could be obtained satisfactorily, but results were generally unsatisfactory. When a test of quantitative recovery of *n*-butyraldehyde-2,4-dinitrophenylhydrazone from one of the more satisfactory samples gave only 82% compared with 98 to 100% which is obtained from good adsorbents, further investigation was made on silicic acid from other sources.

SAMPLES OF SILICIC ACID ESPECIALLY PREPARED

Investigations which were made of the ordinary Mallinckrodt reagent grade silicic acid showed that the original material was not suitable for chromatographic purposes, and with ball-milling, etc., the properties could be improved somewhat but a satisfactory formaldehyde-acetaldehyde separation could not be achieved. A special, finely divided silicic acid from the Mallinckrodt Chemical Co. gave very slow rates of filtration even when mixed 1 to 1 with Celite 535 but gave good separations in both the formaldehyde-acetaldehyde and glyoxalmethylglyoxal tests. Nothing further is known about the history of this sample.

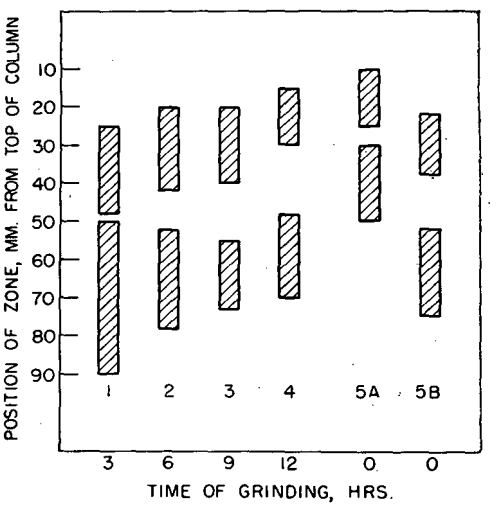


Figure 2. Zone positions with variations in particle size of adsorbent

Test of Mallinckrodt Ramsay and Patterson silicic acid with separation of dinitrophenylosazones of glyoxal and methylglyoxal. Original material and samples ball-milled and mixed with Celite
1 to 4. Silicic acid mixed with 0.5 part of Celite 535
5*A* to 5*B*. Silicic acid as received: no Celite
1 to 5*A*. Identical procedure of development
5*B*. Additional development to improve comparison

When the Mallinckrodt reagent grade silicic acid which is specified as "Prepared for chromatographic purposes according to the method of Ramsay and Patterson" was studied, very promising results were obtained. The "chromatographic purposes" referred to are for the partition methods developed by Ramsay and Patterson (*3*), and the particle size of the material as sold is convenient for that purpose. The material referred to hereafter as "chromatographic silicic acid," when used as received for adsorption chromatography gives a satisfactory flow rate, but the extrusion characteristics and the occurrence of a certain amount of distortion leave room for improvement. In addition, when the material can be mixed with an inexpensive bulky material such as Celite 535, the cost is considerably reduced. When some experiments on ball-milling showed that considerable improvement in separations and in these characteristics would result, an investigation was made to determine the optimum conditions.

The variations in particle size in different lots of silicic acid are shown in Table II; the effect of the grinding procedure is also shown.

The chromatographic properties of this adsorbent as received and after certain procedures of grinding were tested with the formaldehyde-acetaldehyde and glyoxal-methylglyoxal pro-

Table II. Particle Size Characteristics of Chromatographic Silicic Acid as Received and after Grinding

(Percentage particle size classified with U. S. standard sieves)

Mesh Size	Lot 1	Lot 2	Lot 2 After Grinding, Hours 4	Lot 2 After Grinding, Hours 8
+80	7	3	0	0
−80, +150	16	12	0	0
−150, +200	11	11	0	0
−200, +300	17	19	5	0
−300	49	55	95	100

cedures; the results are presented in Figures 1 and 2. The silicic acid as received, without grinding or mixing with Celite, can be used. The very much sharper zones in the samples ground and mixed with Celite, the easier extrusion, lower cost, and other factors all indicate that the work of grinding the adsorbent is justifiable where chromatographic work of any difficulty is encountered. The amount of grinding must be selected by balancing the slight improvement in separations between for example the 9-hour and 12-hour samples against the very much slower rate of flow with the finer grinding.

A procedure for grinding chromatographic silicic acid (*3*) for 8 to 10 hours, mixing with 0.5 part of Celite 535, and heating for 4 hours at 200° C. has been adopted for the author's chromatographic work. With this adsorbent, very good separations have been attained in work which has included a very wide variety of functional groups and organic molecules.

The unsatisfactory results with the earlier samples of ordinary silicic acid show that particle size of an adsorbent is not the sole critical factor. However, the performance of a material which has suitable adsorption properties can be improved by decreasing the particle size.

LITERATURE CITED

(1) Malmberg, E. W., *J. Am. Chem. Soc.*, **76**, 980 (1954).
(2) Malmberg, E. W., Trueblood, K. N., and Waugh, T. D., ANAL. CHEM., **25**, 901 (1953).
(3) Ramsay, L. L., and Patterson, W. I., *J. Assoc. Offic. Agr. Chemists*, **28**, 644 (1945).

RECEIVED for review June 29, 1954. Accepted November 10, 1954.

An Improved Acidimetric Determination of Fluoride

J. M. CHILTON and A. D. HORTON

Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

In an unbuffered solution, the titration of a neutral solution of fluoride with aluminum ions results in an abrupt change of pH at the stoichiometric end point. Details of the separation of fluoride from interfering substances are presented; only phosphate and highly associated fluoride complexes require distillation. With the outlined procedure, in which a recording pH meter was used, fluoride was determined in a sodium fluoride solution with a standard deviation of less than 0.2%, and in a fluosilicate solution with a standard deviation of 0.6%. Analysis of four samples of NBS phosphate rock No. 120 yielded an average recovery of 99.1%. The method is applicable in the range of 0.1 to 3.5 mg. of fluoride per ml. of water.

THE determination of fluoride by distillation from a perchloric acid solution, followed by the titration of the fluosilicic acid with thorium nitrate in a buffered solution, was introduced by Willard and Winter (*9*). However, when performed by an inexperienced analyst, this procedure lacks the precision which a recording instrument will provide.

All proposed methods for the determination of fluoride, both volumetric and colorimetric, depend on the reaction of the fluoride with a metal ion, such as thorium, zirconium, iron, or aluminum, to form a highly associated complex. The colorimetric procedures depend on the determination of the amount of the metal in excess of that complexed by the fluoride. In the Willard-Winter titration, the appearance of an excess of thorium is indicated by the formation of a lake. A procedure by Willard and Horton (*8*) modifies this to determine the appearance of an excess of thorium by a fluorescent indicator.

Greeff, in 1913, proposed the titration of fluoride with ferric iron, using thiocyanate as an indicator (*2*).

$$F^{+++} + 6F^- \rightarrow FeF_6^{---} \quad (1)$$

He also proposed the titration of fluosilicic acid with standard hydroxide

$$H_2SiF_6 + 2KOH \rightarrow K_2SiF_6 + 2H_2O \quad (2)$$

In 1926, Treadwell and Kohl titrated fluoride with both aluminum and iron, using a potentiometric indication of the ferrous-ferric couple as an end-point indicator (*7*).

$$FeF_6^{---} + Al^{+++} \rightarrow AlF_6^{---} + Fe^{+++} \quad (3)$$

In 1930, Kurtenacker and Jurenka investigated several reagents for the titration of the fluoride ion, using pH indicators to detect the end point (*3*). Among the titrants used were boric acid, cerous nitrate, and aluminum chloride. Aluminum ions, added to a neutral solution of fluoride ion, react with the fluoride to form a complex anion.

$$Al^{+++} + 6F^- \rightarrow AlF_6^{---} \quad (4)$$

Any excess aluminum which is added to the solution will hydrolyze, causing an increase in hydrogen ion concentration.

$$Al^{+++} + n(OH)^- \rightarrow Al(OH)_n^{3-n} \quad (5)$$

Batchelder and Meloche made further investigations with the cerous titration, using methyl red as an indicator, and concluded that the color change at the end point was an adsorption phenomenon (*1*).

All of these methods were checked to determine which was best adapted to use with a recording, automatic titrator—e.g., Dow-Precision recording titrator. The titration of fluosilicic acid with standard base was found to be unsatisfactory because the reaction is not instantaneous and does not give a sharp break unless the titration is performed very slowly, and the presence of other strong acids in the sample interferes. Another hydroxide reaction, given by Treadwell (*6*), is also very slow, and seems to go to completion only in the presence of an excess of hydroxide.

$$SiF_6^{--} + 6OH^- \rightarrow 6F^- + Si(OH)_4 + 2H_2O \quad (6)$$

Solutions of reagent grade sodium fluoride were titrated with a solution of reagent grade potassium alum. Both the procedure

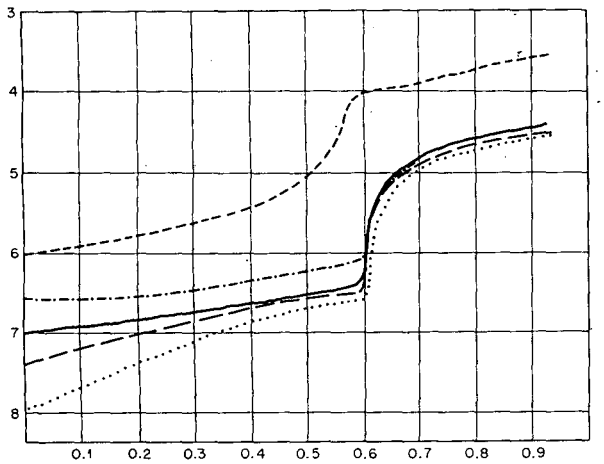


Figure 1. Effect of starting pH on titration of 5.0 mg. of fluoride

of Treadwell and Kohl, using the ferrous-ferric couple, and the procedure of Kurtenacker and Jurenka, using pH change, were tried under identical conditions. The pH change at the stoichiometric end point was found to be more abrupt than the ferrous-ferric potential change. Although the sharpness of the latter break could be increased by making the titration very slowly, the pH break proved more satisfactory.

INTERFERENCES AND SEPARATIONS

Interferences in this method may be divided into two groups: ions of weak acids or bases which exert a buffering action between pH 7 and 4, and ions which react with the fluoride to form undissociated complexes or insoluble precipitates.

In the first group are included ammonium, bicarbonate, and phosphate ions. Ammonium ions can be removed by heating the solution with excess sodium hydroxide. Carbon dioxide can be removed by lowering the pH of the solution to 1.0 and stirring rapidly for several minutes. The sample is then neutralized again with carbonate-free sodium hydroxide. Phosphate cannot be conveniently separated and requires a perchloric acid distillation.

In the second group are a number of metallic ions, including ferric, uranyl, ceric, aluminum, thorium, lanthanum, calcium, barium, and lead. Many of these could be separated without a distillation by the precipitation of the metal with sodium hydroxide and determination of the fluoride in the supernate. This method proved adequate in the case of uranium, iron, copper, cobalt, cerium, and lanthanum (Table I). The presence of other metals, such as lead, aluminum, and calcium, requires a perchloric acid distillation. Boron, when present as borate ion or as boron trifluoride, does not interfere. However, Shell and Craig (*5*) have shown that acid solutions containing borate and fluoride, when subjected to heat, form a nonionized compound of unknown composition. This fact was borne out in this work, in which certain samples containing known amounts of fluoride and large amounts of borate were titrated, and only 30 to 40% of the fluoride was recovered. The technique proposed by Shell, fusion with sodium carbonate, proved effective and all of the fluoride was recovered in these samples after such a fusion. Thorium also can be separated by fusion with sodium carbonate, followed by a filtration through Whatman No. 2 filter paper.

Perchloric acid distillation converts fluoride to fluosilicic acid. This can be reconverted to fluoride by treatment with hydroxide (Equation 4). Although this reaction is slow at room temperature, at 50° to 60° C. completion is within 5 to 10 minutes. The breaks obtained in the titration of fluosilicic acid samples are usually not so abrupt as in the titration of pure sodium fluoride samples, but are generally adequate. This is thought to be due primarily to traces of carbonate in the hydroxide used for neutralization, since the same effect is observed when excessive amounts of strong acids, such as nitric acid, are present in undistilled samples.

EXPERIMENTAL

The sharpest breaks occur in this titration only when the starting solution has a pH above 6.0. At pH 8.0 and above, the break comes after the stoichiometric end point. The best starting pH is 7.0 ± 0.5 (Figure 1). Identical samples were run, adding an equal volume of 95% ethyl alcohol to one aliquot and not to the other. The sample containing the alcohol gave a better break (Figure 2). Acetone works equally as well.

The sharpness of the break at the end point as a function of concentration of fluoride in the aqueous solution was investigated. Because of the limited solubility of potassium alum in water, the greatest concentration of fluoride that can be obtained at the end point is about 3.5 mg. per ml. of water. The lowest concentration of fluoride at the end point which gives a sharp break is approximately 0.1 mg. of fluoride per ml. of water. Concentrations as low as 0.05 mg. per ml. have been titrated, but with some loss in precision (Figure 3). Roden, citing work by West and Watters (*4*), mentions a similar procedure in which thorium nitrate is used as a titrant. By using the mid-point of a

Table I. Effect of Foreign Ions on Fluoride Determination

Ion Added to F Soln.	Moles Added per Mole F	Mg. Fluoride Found 1.00	5.00	10.00	Error Av., %	
NH_4^+	1	..	5.02	10.06	+ 0.5	
	10	1.04	5.21	10.34	+ 4	
	20	..	6.13	...	+23	
HCO_3^-	1	1.10	5.13	...	+ 0.12[a]	Very poor break
PO_4^{---}	0.05	..	5.15	...	+ 3	Poor break
	0.1	0.98	..	...	− 2	
	0.5	..	5.59	...	+12	Very poor break
	1	?	?	...		No definite break
SO_3^{--}	0.5	..	5.07	...		
	1	1.00	..	...	0	
	3	0.99	5.08	...		
SO_4^{--}	0.5	..	5.06	...		
	1	..	5.05	...	0	
	2.5	..	4.96	...		
	5	..	4.94	...		
BO_3^{---} (or H_3BO_3)	0.5	1.02	4.96	...	0	Poor break
BO_3^{---}	1	0.99	4.98	...	− 0.02[a]	Supernate from fusion
Cu^{++}	0.15	0.97	5.28	10.15	+ 2	No sepn.
	1.5	0.99	5.27	?		
Co^{++}	0.5	1.02	5.17	...	+ 2	No sepn.
Zn^{++}	0.5	0.99	5.02	...	0	No sepn.
Ca^{++}	0.05	?	?	...		No definite break
	0.5	..	?	?		Supernate from fusion
Pb^{++}	0.5	..	3.23	...		Poor break
	1	?	..	?		No definite break
Fe^{+++}	0.05	..	5.04	9.90	0	Supernate from pptn.
	5	0.98	5.07	...		
La^{+++}	0.05	..	5.04	9.94	0	Supernate from pptn.
U^{+6}	0.1	..	5.04	...	− 0.5	Supernate from fusion
	1	0.97	..	10.06		
Ce^{+4}	1	..	4.98	10.02	0	Supernate from fusion
Ce^{+++}	0.5	..	5.20	9.64	+ 0.2	Poor break
Th^{+4}	0.5	1.02	4.78	10.22	0	

[a] In milligram.

very gradual break as an end point, concentrations of pure fluoride as low as 0.015 mg. per ml. were titrated.

PRECISION AND ACCURACY

Samples containing 5.0 mg. of fluoride in approximately 5 ml., both as sodium fluoride and as fluosilicic acid were titrated by this procedure to establish the precision. Titration of samples of pure sodium fluoride showed a standard deviation of less than 0.2%, and samples containing fluosilicate showed a standard deviation of 0.6%. Accuracy of the method was established by determination of fluoride content of four samples of NBS phosphate rock standard No. 120. The amount of fluoride titrated, after distillation with perchloric acid, was 98.6, 98.2, 99.0, and 100.5%; an average of 99.1%.

Solutions of sodium fluoride and potassium alum were prepared from reagent grade chemicals, the sodium fluoride being dried at 110° C. These were found to react exactly in the theoretical ratio—one mole of aluminum equivalent to 6 moles of fluoride.

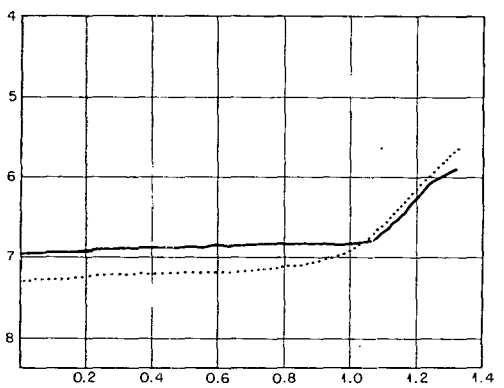


Figure 2. Effect of alcohol on fluoride titration

—— With alcohol
.... Without alcohol

All attempts to carry out the titration in the reverse direction failed; probably due to the stepwise formation of the aluminum fluoride complex.

PROCEDURE

Samples Requiring Perchloric Acid Separation. Take an aliquot of the sample to contain approximately 5.0 mg. of fluoride. Distill according to the Willard-Winter procedure, keeping the distillate barely basic to phenolphthalein with sodium hydroxide. In a platinum crucible, evaporate the distillate to a volume of 2 to 3 ml., or until salts begin to precipitate. Transfer to a small beaker, using as little water as possible. Place in the beaker a magnetic stirring bar and the glass-saturated calomel electrodes leading to an automatic titrator or other pH meter, such as Beckman Model G. Add concentrated hydrochloric acid to lower the pH to 1.0 or less. Saturate the solution with sodium chloride and add 2 to 3 grams in excess. Stir vigorously about 5 minutes. Add an equal volume of 95% ethyl alcohol or two thirds of the volume of acetone. With a heat lamp, raise the temperature to 50° to 60° C., then add carbonate-free 1*N* solution of sodium hydroxide to bring the pH to approximately 7.0. Several minutes may be required to establish pH equilibrium. When the pH remains constant for approximately 45 seconds, use 0.01*N* sodium hydroxide or hydrochloric acid as necessary to bring the solution to pH 7.0 ± 0.5. Remove the heat lamp, and titrate with a standard solution of potassium alum, recording the pH during the course of the titration. The end point is taken as the point of inflection of the curve.

Samples Requiring Hydroxide Precipitation of Metals. Take an aliquot of sample to contain approximately 10 mg. of fluoride. Add 1*N*/sodium hydroxide until basic to phenolphthalein, then 1 ml. in excess. If necessary, concentrate to less than 10 ml. in a platinum dish. Stir with magnetic stirrer several minutes.

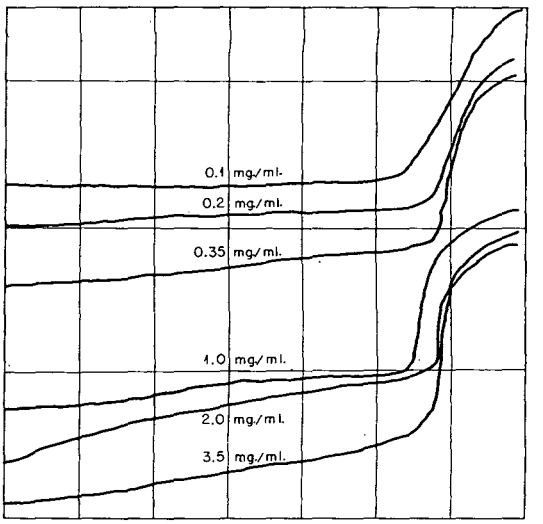


Figure 3. Titrations of various concentrations of fluoride solutions

(Samples containing cerous ions require 0.5 hour or longer.) Transfer to a graduated, 10-ml. centrifuge cup, make to volume, and centrifuge until the supernate is clear. Pipet 5.0 ml. of the supernate and proceed with the addition of hydrochloric acid.

Samples Containing Boron. If there is reason to suspect that a nonionized compound of boron and fluorine is present, transfer an aliquot of the sample to a platinum crucible, and make basic with sodium carbonate. Evaporate to dryness, add 1 gram of sodium carbonate, and fuse at 875° C. Dissolve the fusion in as little water as possible, transfer to a small beaker, and proceed with the addition of hydrochloric acid.

Calculation. One mole of aluminum (26.98 grams) is equivalent to 6 moles of fluoride (114.0 grams).

DISCUSSION

This procedure, though based on that of Kurtenacker and Jurenka (*3*), offers the following advantages over the original:

Use of potassium alum rather than aluminum chloride eliminates the necessity of a tedious neutralization of the titrant.

Reagent grade potassium alum serves as a standard, eliminating the necessity of indirect standardization.

Addition of acetone or alcohol to the solution yields sharper pH breaks.

Use of a recording pH meter gives much more precision than use of a colorimetric indicator.

The proper execution of this procedure requires some experience on the part of the analyst. The precautions necessary to ensure that no carbon dioxide is present in the sample when titrated should not be minimized.

LITERATURE CITED

(1) Batchelder, G., and Meloche, V. W., *J. Am. Chem. Soc.*, 53, 2131 (1931).
(2) Greeff, A., *Ber.*, 46, 2511 (1913).
(3) Kurtenacker, A., and Jurenka, W., *Z. anal. Chem.*, 82, 210 (1930).
(4) Rodden, C. J., ed., "Analytical Chemistry of the Manhattan Project," p. 237, McGraw-Hill, New York, 1950.
(5) Shell, H. R., and Craig, R. L., ANAL. CHEM., **26**, 996 (1954).
(6) Treadwell, F. P., and Hall, W. T., "Analytical Chemistry," **9th** ed., **II**, p. 511, Wiley, New York, 1942.
(7) Treadwell, W. D., and Kohl, A., *Helv. Chim. Acta*, 9, 470 (1926).
(8) Willard, H. H., and Horton, C. A., ANAL. CHEM., **22**, 1190 (1950).
(9) Willard, H. H., and Winter, O. B., IND. ENG. CHEM., ANAL. ED., 5, 7 (1933).

RECEIVED for review August 16, 1954. Accepted November 23, 1954. Presented before the Southeastern Meeting of the AMERICAN CHEMICAL SOCIETY, Birmingham, Ala., October 21, 1954.

Determination of Impurity with the Melting-Temperature Apparatus of Smit

LEE T. CARLETON

Aerojet-General Corp., Azusa, Calif.

A quantitative evaluation of impurity in samples of less than 0.5 gram is described. The method depends on a determination of the melting-temperature curve in a simple, inexpensive apparatus. Tests with samples of known composition indicate that results are probably accurate within 10 to 30% of the total impurity.

THIS paper describes a simple experimental method for the determination of impurity from melting-temperature curves. The method is restricted to samples which are nearly pure and in which, on freezing, the pure major component solidifies, leaving the impurity in solution.

The temperature of equilibrium in such a system is expressed by

$$N_2 = \frac{\Delta H}{RT^2}(T_{fo} - T) = A(T_{fo} - T) \qquad (1)$$

where N_2 is the mole fraction of solute, ΔH is the heat of fusion of solvent, T_{fo} is the freezing point of pure solvent, and T is the equilibrium temperature.

This equation has several different uses depending on which of the variables is to be evaluated. Thus, in the cryoscopic determination of molecular weight, the difference $T_{fo} - T$ is measured for addition of solute, and molecular weight is determined from the relation of N_2 to the weight added. Or, for control of the quality of successive preparations of a single compound for which A and T_{fo} have been previously established, a series of measurements of T yields corresponding contents of impurity expressed as values of N_2. These two uses presuppose a known value of T_{fo} and entail only a single measurement of temperature—i.e., the temperature of equilibrium between the solution and a small amount of frozen solvent. However, the author presents a third use which is different with respect to the data required.

It is necessary, at times, for a laboratory to determine the purity of a new compound whose properties have not yet been established. For such a compound, if T_{fo} is unknown, the relationship of T to the amount of frozen solvent over the range from complete solid to complete liquid may be used to give the amount of impurity as the limiting value of N_2 (*14*). The relationship of equilibrium temperature to percentage frozen can be determined calorimetrically (*2, 4*). Here ΔH is determined directly in the course of the experiment, but this approach is too difficult for routine use. An alternative is to obtain a curve of temperature *vs.* time for freezing or melting, under conditions in which the amount frozen or melted varies in a known manner with time (determining ΔH in a separate experiment, or estimating it). The methods used have usually been qualitative (*1, 8*); if quantitative, difficult and expensive instruments are required (*3, 5, 7, 11*). There is a need for a simple means for determining degree of purity quantitatively from temperature *vs.* time curves.

The method described, based on the apparatus of Smit (*9, 10*), fills this need. The method combines many advantages which make it particularly suitable for the small laboratory. Less than 0.5-gram sample is required. The apparatus is easily constructed from simple materials, a mercury thermometer graduated in tenths of a degree and a glass sample tube blown to close tolerances. Dimensions of the sample tube ensure maintenance of the equilibrium temperature, which eliminates the need for stirring. A melting-temperature curve may be determined instead of a freezing-temperature curve, and the complications of supercooling are avoided. The rate of heat input to the sample is kept constant by maintaining a constant temperature difference between sample and bath; thus, the fraction melted is a linear function of time, and analysis of the temperature *vs.* time curve is easy.

An analysis of the procedure is presented in a comparison of determined and actual amounts of impurity. These results represent the accuracy of the method. This accuracy is satisfactory for many applications, but should not be compared with the accuracy obtained by the procedure of the Bureau of Standards (*3, 5*).

APPARATUS

The enclosure of the sample in this arrangement is in the form of a thin, uniform film surrounding the bulb of a mercury thermometer. Smit gives an extensive analysis of the effect of dimensions on performance of the apparatus, which requires a reasonable speed of heating (0.3° C. per minute before melting), and uniformity of temperature throughout the sample. These requirements lead to the following optimum conditions:

Rate of heating, ° C./min.	0.3
Inside radius of sample tube, cm.	About 0.35
Wall thickness of sample tube, mm.	About 0.4
Thickness of layer of sample, mm.	About 0.7
Sensitivity of thermometer, ° C.	0.01

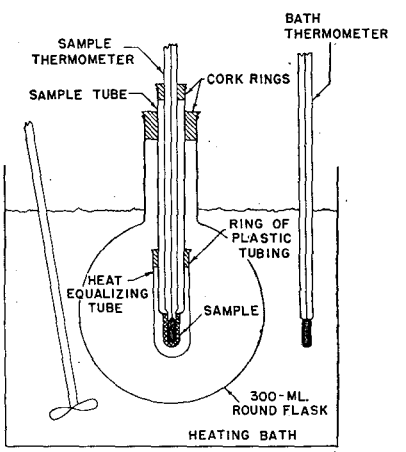


Figure 1. Apparatus for determination of melting temperature

Based on these conditions, the simple apparatus shown in Figure 1 was constructed. A partial-immersion thermometer graduated in tenths of a degree is positioned, by means of a bored cork, in a glass sample tube drawn to the proper dimensions in the portion surrounding the thermometer bulb. To diminish the effects of temperature fluctuation, the sample tube is jacketed with a slightly larger tube retained by a ring of plastic tubing. Following the arrangement of Malotaux and Straub (*6*), this assembly is mounted with a larger bored cork in a 300-ml. round-bottomed flask, in such a position that the thermometer bulb is approximately at the center of the flask. The flask is immersed to the neck in a suitable heating bath which is provided with a stirrer, a sensitive manual control of temperature, and a thermometer similar to the sample thermometer.

Choice of the sample thermometer to be used depends on the sensitivity requirement. The range should be such that all temperatures to be read lie above the retaining cork, and the graduations, should be spaced widely. Under these conditions temperature differences of 0.01° C. may be distinguished with the aid of a magnifying glass.

PROCEDURE

The sample tube is filled with enough molten sample to rise about 2 mm. above the thermometer bulb when it is in place.

(About 0.3 ml. will be required.) The thermometer bulb is inserted into the molten sample and carefully adjusted into a position of symmetry. Then the sample is frozen, and the apparatus is assembled as shown in Figure 1.

Beginning at a temperature about 30° C. below the melting point, the bath is heated at a rate of 0.3° C. per 100 seconds. (This rate was adopted after some earlier experiments with rates of about 0.3° C. per minute.) After a few minutes, the sample thermometer will have assumed a temperature which differs from the bath temperature by a constant amount (about 2° to 3° C.). This constant differential is maintained within ±0.1° C. throughout the heating period. A plot of sample temperature *vs.* time is started at 15° to 20° C. below the melting point. Three minutes is a convenient interval between readings; both sample and bath temperatures are recorded, even though only the sample temperature is plotted.

With the onset of melting and the flattening of the curve, the bath heat must be lowered in such a way as to maintain the constant temperature differential. The curve rises more steeply when melting is completed; again, the heat must be adjusted to maintain the constant differential. The experiment should be continued until enough points have been taken to define the straight line which represents heating of the completely liquefied sample.

After the run is complete the equilibrium curve is drawn on the completed plot. Up to the time when more than one half of the sample has been melted, this curve is a smooth line through the points. During the last part of the melting process there is a wide departure from equilibrium, so that the curve is flattened below the experimental points, instead of bending upward with them. (Observations in a glass-walled bath show that, in the last stages of melting, the remaining crystals are loosened and fall to the bottom of the tube. The liquid is no longer in close contact with solid throughout the sample, so that superheating is possible.) The curve is then extrapolated to intersect the downward projection of the liquid heating line; the temperature at this intersection is taken as a first approximation of T_o, the freezing point of the sample. The T_o, the freezing point of the entire sample containing both major component and impurity, is to be distinguished from T_{fo}, the freezing point of the pure major component.

This temperature must be readjusted to its final value by estimating the total time of the melting process, taking the amount of melting as proportional to the time, and measuring values of ΔT (below the first-approximation freezing point) corresponding to one half, one third, one fourth, etc., of the sample melted. A plot of ΔT against the reciprocal of the fraction melted, $1/m$, will then yield a straight line. This line will cross the horizontal axis at a value of $1/m$ equal to unity if the freezing point has been chosen correctly. If it has not, the temperature should be changed to a final value for which the line does pass through this point. The final value of T_o determined by this procedure should be correct within ±0.025° C. This line may also be used to estimate x, the mole fraction of impurity, as the product of A and the slope. [Alternatively, the method of Taylor and Rossini (*12*) may be used to determine T_o.]

ANALYSIS OF TEMPERATURE *VS.* TIME CURVE

Analysis of the resulting temperature *vs.* time curve is based on the linear relationship of heat input to time and on Equation 1. The ideas underlying all such treatments are clearly pointed out by White (*14*).

The linear relationship of heat input to time follows from the maintenance of a constant temperature interval between the sample and bath. During melting this heat input has two components: a specific-heat component which raises the temperature of sample and thermometer bulb; and a melting component.

To analyze the temperature *vs.* time curve, these two components must be resolved and the melting component must be related separately to the time scale. The procedure is illustrated in Figure 2, where the solid line is an actual equilibrium curve, and the dashed lines are the separated components of heating and melting.

This example is taken from a two-component system. The initial straight portion slanting upward, PQ, represents the heating of solid sample and the thermometer bulb. In this particular case, there is a definite flattening at the eutectic melting temperature, QR. (In many actual cases, however, there is more than one impurity and this characteristic is obscured.) Above this eutectic, melting and heating occur simultaneously, RS; the

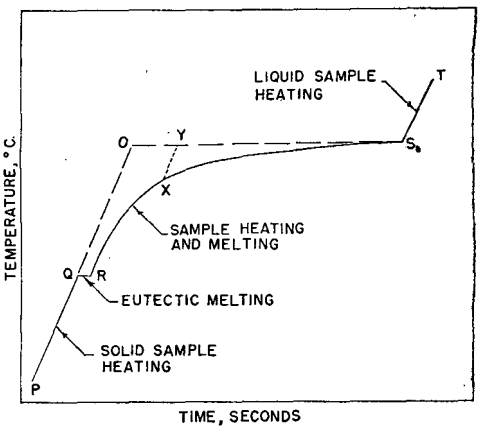


Figure 2. Melting-temperature curve for two-component system

——— **Actual equilibrium curve**
– – – – – **Idealized process**
------- **Heating without melting (specific-heat component)**

curve arches and flattens until all solid is melted. The slope changes abruptly when heating of the completely liquefied sample begins, ST.

The dashed line represents an idealized process in which all solid is heated to the freezing point and then all the sample is melted isothermally. The two lines, QO and OS, are thus separate specific-heat and melting components for the actual process. The flat line, OS, represents the melting of two (or more) substances, solvent and solute, with different heats of fusion. However, in this work the amount of solute is always small, so that on the central part of the curve, which is used for analysis, the fraction of material melted is substantially proportional to the distance along OS.

At a point X on the actual curve, from which the dotted line XY to the ideal flat line represents heating without melting, the fraction melted is OY/OS.

Analysis of an experimental curve depends on the construction of several projections, such as XY, from the actual curve to the ideal flat line. The same slope as that of PQ could be used with a negligible error (due to the difference in specific heats of solid and liquid sample). However, a run usually begins at a temperature at which some melting is already under way, so that the slope of the separate specific-heat component is not known.

When the properties of the sample are known, a slope for XY may be approximated from the dimensions of the apparatus, the specific heat and heat of fusion of the sample, and a rough estimate of the amount of impurity, assuming ideal solution behavior. Usually, these properties of the sample will not be known, but either the properties or the slope may be estimated with sufficient accuracy. For the portion of the curve used for analysis, the length XY is short and a crude estimate of the slope suffices.

This discussion shows how to obtain the line XY for any point X, and the appropriate differences of temperature, ΔT, and time, $\Delta t(=YS)$, from the reference point S, but does not show how these data may be used to calculate the amount of impurity present.

If x represents the mole fraction of impurity in the original sample and T_o the freezing point of the impure sample (the temperature at point S on Figure 2), then Equation 1 gives

$$N_2 = A(T_{fo} - T) \tag{2}$$

$$x = A(T_{fo} - T_o) \tag{3}$$

whence

$$N_2 = x + A(T_o - T) \tag{4}$$

If f is the mole fraction of the solvent frozen at temperature T,

$$f = \frac{N_2 - x}{N_2} = \frac{A(T_o - T)}{x + A(T_o - T)} = \frac{A\Delta T}{x + A\Delta T} \quad (5)$$

If Δt represents the time difference from the point of complete melting (length YS on Figure 2) and Δt_o the total time represented by the ideal-melting flat line (length OS), then

$$f = \frac{\Delta t}{\Delta t_o} \quad (6)$$

Equations 5 and 6 are rearranged

$$\Delta t = \Delta t_o - \frac{x}{A}\frac{\Delta t}{\Delta T} \quad (7)$$

Thus, theory predicts that a plot of Δt against $\Delta t/\Delta T$ gives a straight line whose slope is $-x/A$. The term A is equal to $\Delta H/RT^2$ and is a characteristic property of the major component. When ΔH is not known, A may be determined from an additional run on the sample containing a known mole fraction of added solute. Alternatively, ΔH may be estimated by comparing the curve for the sample with a curve for a reference substance of known heat of fusion, obtained in the same apparatus (*5*). Then x is the product of A and x/A determined from the slope.

TEST OF THE METHOD

The method was tested with synthetic samples from the following systems:

C.P. naphthalene + anthracene (minor component)
C.P. naphthalene + diphenyl (minor component)
NBS benzoic acid (standard sample of 39 grams and certified purity of 99.99% by titration) + anthracene (minor component)

The second is known to form an ideal system (*13*). Heats of fusion and specific heats for both naphthalene and benzoic acid are known accurately.

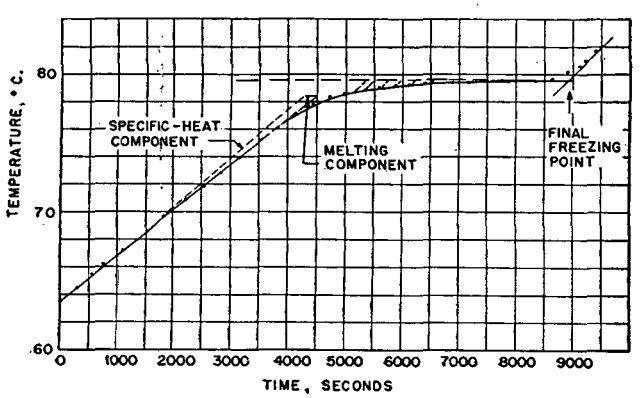


Figure 3. Melting-temperature curve, C.P. naphthalene

For example, melting-temperature curves for C.P. naphthalene alone and for C.P. naphthalene containing 1.45 mole % diphenyl are analyzed. The original curves are shown in Figures 3 and 4 with the ideal-melting flat line extended across from T_o, and diagonal lines representing heating without melting (specific-heat effect) drawn in at selected values of ΔT. Slopes of these lines were obtained by resolving the slope of the equilibrium curve at 70° C. into separate specific-heat and melting components (calculated from the dimensions of the apparatus and the properties of naphthalene). This calculation is as follows for C.P. naphthalene.

T_o and x were estimated as 79.7° C., and 0.003, respectively, by the method described. In the 10° C. temperature interval from 65° to 75° C., the change in the fraction melted, $\Delta(1 - f)$, was calculated from

$$\Delta(1 - f) = \Delta\left(\frac{x}{N_2}\right) = \Delta\left(\frac{x}{x + A(T_o - T)}\right)$$

Because A is equal to 0.0184 for naphthalene, $\Delta(1 - f)$ is equal to 0.0226. The melting component for the 65° to 75° C. temperature interval is the product of 0.0226 and 35.6 calories per gram (the heat of fusion for naphthalene), or 0.81 calorie per gram. The specific-heat component is the number of calories needed to heat 1 gram of naphthalene plus the corresponding surrounded volume of the thermometer bulb through the same temperature interval. (The thermometer bulb is considered to be all mercury.) From the dimensions of Apparatus II, for each gram of naphthalene (0.91 ml.) of specific heat 0.358 calorie per gram, there is 1.71 ml. or 22.9 grams of mercury of specific heat 0.033 calorie per gram and the total value of the specific-heat component is $10[(1 \times 0.358) + (22.9 \times 0.033)]$, or 11.1 calories per gram.

A triangle was constructed with the slope of the equilibrium curve at 70° C. forming the long side (Figure 3). The other two sides represent the melting and specific-heat components; their lengths are in the ratio 0.81 to 11.1. The slope of the specific-heat component obtained from this triangle is used in the analysis.

Lines having the slope of the specific-heat component (corresponding to XY of Figure 2) were drawn for selected values of ΔT, and the intersections with the ideal-melting flat line gave the corresponding values of Δt. Table I gives these values of ΔT, Δt, and $\Delta t/\Delta T$, both for the C.P. naphthalene and for C.P. naphthalene plus 1.45 mole % of diphenyl.

A reference temperature interval in which little melting takes place is chosen so that the melting component is small in comparison with the specific-heat component. In analysis of the equilibrium curve the steepest portion should be avoided, because in this portion Δt changes rapidly with a small change in slope; thus, any error introduced in the choice of slope must be slight.

Figures 5 and 6 show the plots of Δt against $\Delta t/\Delta T$. The best straight lines have been drawn and their slopes are −0.172 and −0.95, respectively. From the known heat of fusion of naphthalene, A is 0.0184. Hence, for C.P. naphthalene, $x = 0.0032 = 0.32$ mole %, and for naphthalene-diphenyl, $x = 0.0175 = 1.75$

Table I. Illustrative Values of $\Delta t/\Delta T$ from Curves

Naphthalene				Naphthalene + 1.45 Mole % Diphenyl			
ΔT, ° C.	Δt, sec.	$\Delta t/\Delta T$	f	ΔT, ° C.	Δt, sec.	$\Delta t/\Delta T$	f
0.2	2360	11,800	0.563	1.0	1990	1990	0.522
0.3	2660	8860	0.635	1.5	2330	1553	0.612
0.4	2920	7300	0.697	2.0	2570	1283	0.676
0.5	3120	6240	0.745	2.5	2760	1102	0.725
0.7	3360	4800	0.802	3.0	2910	970	0.765
0.9	3520	3910	0.840	4.0	3160	790	0.830

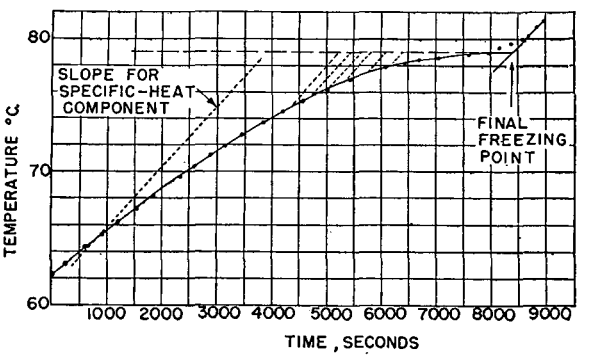


Figure 4. Melting-temperature curve, C.P. naphthalene + 1.45% diphenyl

mole % (total figure including both the added diphenyl and the original impurity).

The results for all mixtures of the three systems are summarized in Table II. The criterion of satisfactory results is agreement between actual and calculated values of Δx, the amount of impurity added. The benzoic acid used contains no detectable impurity; however, tests of the C.P. naphthalene show that an appreciable amount of impurity is present originally in this material, and a correction must be made for this amount in computing Δx. This amount is taken as the average from all the determinations on C.P. naphthalene.

The different pieces of apparatus and different rates of heating used are identified on the table. No one combination appears to be best. However, in general, experience shows that runs made at the lower rate (ca. 0.3° C. per 100 seconds) before melting are more conveniently analyzed, so this rate of heating is recommended.

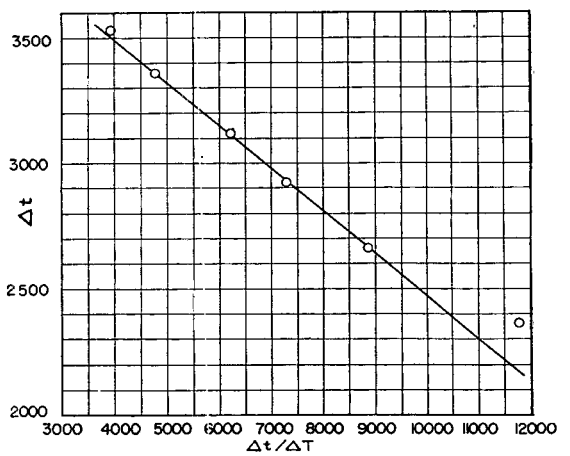


Figure 5. Derived line, C.P. naphthalene

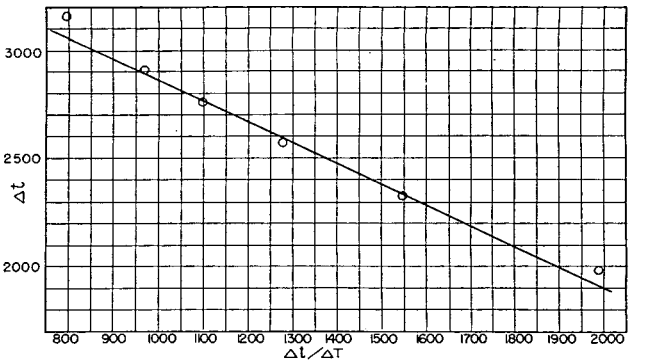


Figure 6. Derived line, C.P. naphthalene + 1.45% diphenyl

ACCURACY AND REPRODUCIBILITY

Application of the method must be restricted to those systems in which pure solvent crystallizes out. For such systems the results in Table II indicate the accuracy and reproducibility obtainable. The highest accuracy and reproducibility, such as obtained by the Bureau of Standards (*3*, *5*), have been sacrificed for simplicity and low cost. With substances of known heats of fusion in the concentration ranges (98.5 to 99.7%) investigated, over-all average results from two or more experiments are probably accurate to within 10 to 30% of the total impurity. As the amount of impurity in the sample increases, the absolute error also increases. The sensitivities of the thermometers used in this investigation do not permit evaluation of high-purity substances, since for such substances the temperature changes on melting are too small to be measured accurately. In unusual cases of contamination of a low-melting major component with a high-melting impurity, the eutectic can lie so close to the former on the phase diagram that Equation 1 does not hold for any appreciable range of concentration; in these cases, the error will be larger. This was shown with the system diphenyl oxide (melting point 27° C.)–anthracene (melting point 217° C.).

Sources of error are coarseness of temperature measurement, and oversimplifications in analyzing the equilibrium curve; assumptions in treatment of heat transfer by which Smit derived the optimum dimensions of the apparatus; and thermodynamic and numerical approximation inherent in Equation 1.

In the first source of error, reproducibility and accuracy possibly could be improved by using more sensitive thermometers. The thermometers used in the tests summarized in Table II were graduated in tenths, and in one instance, fifths of a degree. Therefore, temperature readings are reliable to a few hundredths. The final values of T_0 must be rounded off to within ±0.025° C. When values of ΔT as low as 0.3° C. are used in drawing the straight line, these irreg-

Table II. Comparison of Actual and Calculated Amounts of Impurity

	Description of Apparatus									
	Thermometer									
			Graduations,	Bulb		Sample Tube OD,	Type of Outer Jacket			
No.	Range,	°C.	°C.	Length, mm.	OD, mm.	Mm.				
I	−1 to +201		0.2	25	5.8	7.8	Round-bottomed flask			
II	−1 to +101		0.1	23	5.9	8.1	Round-bottomed flask			
III	−1 to +101		0.1	31	5.9	7.9	Test tube			
IV	−1 to +201		0.2	25	5.8	7.8	Test tube			

Sample as Made Up			Exptl. Conditions		Calcd. Impurity		% Error in Calcd. Impurity
Major component	Contaminant	Δx, %	Apparatus	Speed	x, %	Δx, %	
Naphthalene	....	0.00	II	Fast[a]	0.26		
			II	Fast	(0.53)		
			II	Fast	0.28		
			II	Fast	0.45		
			II	Slow[b]	0.34		
			II	Slow	0.32		
			III	Slow	0.37		
Av.					0.34		
Naphthalene	Anthracene	0.24	II	Slow	0.49	0.15	−38
		0.40	III	Slow	0.67	0.33	−17
		0.89	II	Fast	1.15	0.81	− 9
					1.17	0.83	− 7
Naphthalene	Diphenyl	1.18	II	Fast	1.35	1.01	−14
		1.32	III	Slow	1.53	1.19	−10
		1.45	II	Fast	2.22	1.88	+30
			I	Slow	2.02	1.68	+16
			II	Slow	1.75	1.41	− 3
Benzoic acid	....		I	Fast		Undetectable	
Benzoic acid	Anthracene	0.48	I	Slow	0.83	(0.83)	. .
			IV	Slow	0.37	0.37	−23
Benzoic acid	Anthracene	0.68	I	Slow	0.60	0.60	−12
			IV	Slow	0.55	0.55	−19

[a] Fast, 0.3° C./min. for initial heating period.
[b] Slow, 0.3° C./100 sec. for initial heating period.

ularities may cause some uncertainty in the location of the slope. Generally, however, in determining the slope, deviations from a straight line are systematic rather than random, indicating that the other sources of error predominate.

The last two sources of error are discussed at length in the literature (*5*, *9*, *12*). Their influences are hard to separate. From experience with many runs, equilibrium conditions are believed often to be approximated closely, at least over a sizable portion of the melting process. In particular, the curve for pure benzoic acid showed no temperature change for many minutes; this is good evidence for the validity of Smit's treatment of the heat-transfer problem.

The degree of reproducibility and accuracy obtained suffices for many applications, and the combined errors from all sources (Table II) are usually smaller than the uncertainty introduced by estimating ΔH for a new substance.

LITERATURE CITED

(1) Andrews, D. H., Kohman, G. T., and Johnston, J., *J. Phys. Chem.*, **29**, 914–25 (1925).
(2) Aston, J. G., and Fink, H. L., *Chem. Revs.*, **39**, 357–96 (1946).
(3) Glasgow, A. R., Jr., Streiff, A. J., and Rossini, F. D., *J. Research Natl. Bur. Standards*, **35**, 355–73 (1945).
(4) Johnston, H. L., and Giauque, W. F., *J. Am. Chem. Soc.*, **51**, 3194–214 (1929).
(5) Mair, B. J., Glasgow, A. R., Jr., and Rossini, F. D., *J. Research Natl. Bur. Standards*, **26**, 591–620 (1941).
(6) Malotaux, R. N. M. A., and Straub, J., *Rec. trav. chim.*, **52**, 275–88 (1933).
(7) Schwab, F. W., and Wichers, E., "Temperature: Its Measurement and Control in Science and Industry," pp. 256–64, Reinhold, New York, 1941.
(8) Skau, E. L., *Proc. Am. Acad. Arts Sci.*, **67**, 551–76 (1933).
(9) Smit, W. M., dissertation, Amsterdam, 1946.
(10) Smit, W. M., *Chem. Weekblad*, **36**, 750–2 (1939).
(11) Stull, D. R., IND. ENG. CHEM., ANAL. ED., **18**, 234–42 (1946).
(12) Taylor, W. J., and Rossini, F. D., *J. Research Natl. Bur. Standards*, **32**, 197–213 (1944).
(13) Washburn, E. W., and Read, J. W., *Proc. Natl. Acad. Sci. U. S.*, **1**, 191–5 (1915).
(14) White, W. P., *J. Phys. Chem.*, **24**, 393–416 (1920).

RECEIVED for review March 22, 1954. Accepted October 30, 1954.

Assay of Iron-55 and Iron-59 in Biological Samples

J. H. REDISKE, R. F. PALMER, and J. F. CLINE

Biology Section, Radiological Sciences Department, General Electric Co., Richland, Wash.

A method for determining quantities of iron-55 and iron-59 in a mixture was devised, based on the formation of the iron-55-59 thiocyanate complex and extraction by an isoamyl alcohol and diethyl ether mixture. An aliquot of this organic phase is pipetted onto a stainless steel plate, dried, and counted in a windowless, methane-flow, proportional counter. The ratios of the activities of each isotope are determined by counting through a 0.63 mg. per sq. cm. rubber hydrochloride absorber. This absorber allows 0.66 mg. per sq. cm. of the iron-59 activity to be detected, but only 0.015 of the iron-55 activity.

OF THE radioactive isotopes of iron, iron-59 is perhaps the most commonly used in tracer experiments because of its ease of detection. However, certain inherent characteristics of iron-55, especially the longer half life and softer radiation, have a definite advantage over iron-59 in biological work. Because of the softer radiation, methods for detection of iron-55 in the past have involved either the relatively inefficient process of x-ray detection by a thin window Geiger-Müller tube or the tedious electrodeposition of an infinitely thin sample.

In addition to the use of these isotopes individually, it is often desirable to introduce both iron-55 and iron-59 as a dual tracer in an experiment and determine the activity of each independently. Thus Peacock and coworkers (*2*) were able to measure these two isotopes in blood samples by using electrodeposition for sample preparation with assay on two separate counters. A helium-filled mica window tube was used to detect the iron-59 betas and an argon-filled beryllium window tube was used to detect the soft x-rays from iron-55.

Stewart and Rossi (*6*) also have outlined a procedure for detection of these two isotopes in mixed preparations. The samples are electroplated and counted with a thin window tube. The beta particles of iron-59 are deflected with a magnetic field and the x-rays of iron-55 are allowed to pass unaffected. Corrections are made for the gamma rays of iron-59.

These methods have not proved practical for the particular experiments being conducted at this laboratory, principally because of the tedious electroplating process and the low efficiency with which the iron-55 isotope is detected.

The present work was undertaken as an extension of previous work (*4*) on iron-55. A rapid, sensitive method was developed for detecting the two radioisotopes of iron, either individually or in mixed preparations, which is suitable for analysis of plant tissue. The analysis was carried out by digestion of the plant material with sulfuric and perchloric acids, development of the ferric thiocyanate complex, and extracting this with an organic solvent. An aliquot of the organic phase was evaporated on a stainless steel counting plate to an "infinitely thin" sample. The activity was then determined in a windowless, methane-flow, proportional counter using a 0.63 mg. per sq. cm. absorber to minimize detection of the iron-55.

MATERIALS

Stock solutions of iron-55 and iron-59 used were the standard Oak Ridge catalog items prepared by cyclotron bombardment. The iron-59 solution initially contained <0.1% of iron-55 as determined from decay analysis. The iron-55 stock solution was allowed to decay until any iron-59 which may have been present was reduced to <0.1% of the total activity. This again was confirmed by decay analysis. The activity of each of these stock solutions, as measured in the Nucleometer, was determined individually.

Sample 1(iron-55-59) was prepared as a mixture of aliquots of the two stock solutions. Sample 2(iron-55-59) was prepared independently of 1(iron-55-59), using the same stock solution ratio but a higher total activity. These samples were prepared with dried ground plant material and carried through the described procedures. The isotopes were not allowed to be incorporated into living plants for the control work because of the possibility of isotopic differentiation in the biological processes, thus altering the iron-55 to iron-59 ratio.

The absorber employed was of thin rubber hydrochloride (Reed Laboratories, Inc., Akron, Ohio) mounted on a brass ring designed to fit over the counting plate. The absorber was checked for uniform thickness and found to have a weight of 0.625 mg. per sq. cm.

Radioactivities were determined with a Nucleometer (Radiation Counter Laboratory) using a windowless, methane-flow, proportional counter (R.C.L. Mark 12, Model 1). This instrument is sensitive to iron-59 betas and gammas and appears to be sensitive to iron-55 Auger electrons (*1*). When a sample of iron-55 is covered with the described absorber, only about 1.5% of the total count can still be detected. This is probably result of the iron-55 x-rays.

All reagents were of standard A.C.S. quality.

Table I. Fraction of Activity from Iron-55 and Iron-59

(Detected through a 0.625 mg. per sq. cm. rubber hydrochloride absorber)

Counts per Minute		Fraction
Without absorber	With absorber	Counting through Absorber
	Fe^{59}	
2,115	1397	0.66
2,046	1370	0.67
2,175	1440	0.65
2,130	1386	0.65
4,069	2686	0.66
4,104	2773	0.68
4,278	2779	0.65
4,093	2770	0.68
8,320	5489	0.66
8,359	5407	0.65
8,355	5415	0.65
8,169	5402	0.66
Mean	0.66 ± 0.02 at 90% confidence level	
	Fe^{55}	
40,930	654	0.016
90,413	1265	0.014
125,607	1874	0.015
	Average	0.015

Table II. Determination of Iron-55 to Iron-59 Ratios on Mixtures of Two Isotopes

	Counts per Million				
Solution	Total, T	Through absorber, A	Iron-59 $(A-0.015T)$ (0.645)	Iron-55 $(0.66T-A)$ (0.645)	Iron-55 / Iron-59
1(iron-55-59)	1329	527	786	543	0.69
	1336	535	798	538	0.67
	1328	528	787	539	0.68
	1361	536	800	561	0.70
2(iron-55-59) on 6/9/53	5182	2054	3063	2117	0.69
	5310	2098	3128	2181	0.70
	5578	2253	3362	2213	0.66
	5527	2180	3250	2275	0.70
2(iron-55-59) on 6/24/53	4867	1734	2575	2291	0.89
	4729	1709	2539	2189	0.86

15 days of decay should increase iron-55 to iron-59 ratio to 0.87 on solution 2(iron-55-59).

PROCEDURE

Preparation of Samples. The dried plant material (1 to 2 grams) was placed in a Kjeldahl flask and wet-ashed with concentrated sulfuric acid and enough 60% perchloric acid to clear according to the procedure of Piper (*3*). After clearing, the excess was boiled off from the digest, the flasks were allowed to cool, and the digest was diluted with water to an approximate concentration of 20% sulfuric acid and a known total volume depending on the amount of activity present.

A 1-ml. aliquot of this solution was transferred to a 15-ml. test tube, 10 γ of iron(III) added, and the solution diluted to 10 ml. with water. One milliliter of a 20% potassium thiocyanate solution in water was added and mixed, followed by 2 ml. of a solution made up of equal parts by volume of diethyl ether and isoamyl alcohol which was layered on the aqueous phase. The solution was stoppered, shaken 5 to 10 times, and allowed to stand until the two phases had separated (3 to 5 minutes). A suitable aliquot (0.1 to 1 ml.) of the organic phase was pipetted onto a $1^1/_2$-inch stainless steel counting plate, dried under an infrared heat lamp, flamed lightly over a Bunsen burner, and counted.

Samples prepared according to this technique should result in no visible residue on the plate. Occasionally, however, a black tarry residue appeared. This may have been due to a slight amount of the aqueous phase being transferred to the plates with the organic aliquot. Replating from the organic layer generally resolved this difficulty.

Counting of Samples and Standardization. The sample was counted directly to obtain the total iron-55 plus iron-59 count. It was then counted through a 0.63 mg. per sq. cm. rubber hydrochloride absorber. The counts recorded through the absorber were due principally to iron-59. In Table I, an absorber of this thickness will allow about 1.5% of the iron-55 activity through over a range of activities up to at least 125,000 counts per minute. This factor may vary slightly, depending on the particular absorber and mechanical arrangement used, and should be determined for each individual counting arrangement. This small factor would probably only be significant in mixtures of the two isotopes which are predominantly iron-55. This absorber will also reduce the number of counts from the iron-59, and determination of a factor representative of this reduction was necessary.

Table I shows the results of four individual determinations of this factor at each of three activity levels of iron-59. The fraction of counts, due to iron-59 and detected through the absorber, was the same at the three activity levels, and averaged 0.66 ± 0.02 at the 90% confidence level.

Table II demonstrates the application of this technique to the determination of the ratio of these two isotopes in a mixture. Four independent analyses were made on each of two samples, the plates being counted with and without the absorber. The activity of each isotope was determined as follows:

where

T is total observed counts per minute with no absorber, and A is observed counts per minute through the absorber,

then

$$T = \text{c/m } Fe^{59} + \text{c/m } Fe^{55}$$

$$A = 0.66 \text{ c/m } Fe^{59} + 0.015 \text{ c/m } Fe^{55}$$

From these two basic equations one can derive by algebra:

$$\text{c/m } Fe^{55} = \frac{0.66T - A}{0.645}$$

$$\text{c/m } Fe^{59} = \frac{A - 0.015T}{0.645}$$

Based on the average of these determinations, sample 1(iron-55-59) gave an iron-55 to iron-59 ratio of 0.69. Sample 2(iron-55-59), made up independently at higher concentrations but in the same ratio, also gave an iron-55 to iron-59 of 0.69. This iron-55 to iron-59 ratio is obviously based on the activity as determined in the counter used and is not indicative of the absolute ratio. This ratio is within 5% of the known value based on the activity of the individual isotope solutions employed in preparing the mixture.

To test further the validity of this method, sample 2(iron-55-59) was allowed to decay for 15 days and the iron-55 to iron-59 ratio again determined. The ratio had now increased to an average value of 0.88. A value of 0.87 can be derived from calculating the 15-day effect of decay of iron-59, using 0.69 as the average of the ratio for June 9, 1953.

DISCUSSION

The procedure as outlined has given satisfactory results in many analyses of mixtures of these two isotopes. As applied to a mixture or to each isotope independently, the method is more rapid and sensitive than previously described methods. It has the further advantage that analyses for radioactive iron may be carried out on the same solutions used for the thiocyanate colorimetric analysis of total iron (*5*).

The principal advantages of this method are its speed and simplicity over the time-consuming electroplating method and use of complicated auxiliary equipment to separate the iron-55 and iron-59 activity. The success of this method is due in large measure to the use of the windowless, methane-flow, proportional counter. Its large geometry (near 50%) and sensitivity for soft beta emitters and orbital electron-capture isotopes overcome many of the disadvantages of the thin window Geiger-Müller tube commonly used for these isotopes.

This method has possibilities for other isotopes emitting soft beta particles or decaying by orbital electron-capture. Work is underway at the present time to prepare samples of chromium-51 on plates having a minimum of self-absorption by use of a chromium complex–organic solvent extraction process.

LITERATURE CITED

(1) Miller, F., *Phys. Rev.*, 65, 254 (1944).
(2) Peacock, W. C., Evans, R. D., Irvine, J. W., Jr., Good, W. M., Kip, A. F., Weiss, S., and Gibson, J. G., *J. Clin. Invest.*, 25, 605 (1946).
(3) Piper, C. S., "Soil and Plant Analysis," Interscience, New York, 1944.
(4) Rediske, J. H., U. S. Atomic Energy Commission, AECU-732 (1950).
(5) Sandell, E. B., "Colorimetric Determination of Traces of Metals," Interscience, New York, 1950.
(6) Stewart, W. B., and Rossi, H. H., *Nucleonics*, 11, 66 (1953).

RECEIVED for review May 10, 1954. Accepted November 13, 1954.

Determination of Low Alkalinity or Acidity in Water

T. E. LARSON and LAUREL HENLEY, *Illinois State Water Survey, Urbana, Ill.*

In determining mineral constituents of rain water, a precise determination of extremely low concentrations of alkalinity and acidity was essential. The procedure developed is based on the principle that increments of added acidity (after neutralization) increase the hydrogen ion concentration as a linear function. Extrapolation to 1 × 10^{-7} mole (H^+) per liter provides a precise equivalent end point. The procedure is sensitive and accurate to 0.05 p.p.m. (as calcium carbonate) when titrating a 200-ml. sample of −1 to +1 p.p.m. alkalinity with 0.02*N* sulfuric acid. It might also be used for analysis of deionized water, steam condensate, or air pollution samples.

TITRATIONS of strong and weak alkalies with strong acid have long been used in quantitative analysis for standardization with primary standards and for the routine determination of alkalinities in natural waters (*1*). The use of methyl orange and mixed indicators has proved satisfactory for titration of alkalinity concentrations sufficiently high to ensure a reasonably definite pH at neutralization.

In the analysis of rain water samples in connection with the Cloud Physics Project, sponsored by the U. S. Signal Corps, the concentrations of alkalinity or acidity were found to be low but often a significant portion of the total mineral content. These samples may contain more than 10 times as much carbon dioxide as alkalinity. Therefore, the end point pH is not only variable by definition but unstable owing to loss of carbon dioxide during titration. The difficulty is similar to the problem of the titration to the phenolphthalein end point for free carbon dioxide in waters of very high alkalinity.

Attempts were made to add predetermined concentrations of alkalinity to the samples, but the absolute error introduced by the measurement of added alkalinity and lack of precision in detecting the end point prohibited results of the required precision.

It was then reasoned that if a sample is aerated with carbon dioxide–free gas, the percentage of free carbon dioxide liberated by successive additions of acid would increase as the pH decreased. This was followed by the consideration that following neutralization, successive additions of acid should increase the hydrogen ion concentration (or activity) as a straight line function. Then by extrapolation to zero or to 1 × 10^{-7} mole hydrogen ion concentration per liter, the exact neutralization point could be established.

Calculations revealed that extrapolation to zero rather than 1 × 10^{-7} mole hydrogen ion per liter on a 200-ml. sample would introduce a positive error of only 0.005 p.p.m. alkalinity (Figure 1), well within the desired accuracy and experienced precision.

Calculations further revealed that aeration was not necessary if the titrations were continued to pH values sufficiently low to suppress the ionization of carbonic or other weak acids to an insignificant proportion. For carbonic acid, ionization is suppressed to 10% at about pH 5.36 and 1% at 4.36. Silicic acid is ionized 0.1% at pH 7 and therefore exists essentially as unionized silicic acid at this pH and lower, and causes no interference. For acetic acid the ionization is suppressed only to 10% at pH 3.55 and 1% at 2.55.

The absolute error in hydrogen ion activity, as calculated from a possible error of 0.01 pH unit, increases logarithmically or 10-fold per unit decrease in pH. Therefore, greater accuracy and sensitivity are obtained by intermittent aeration during the titration of bicarbonate alkalinity in order that the extrapolation can be made from maximum values of pH.

Theoretically, the slope of the extrapolation line should correspond to the activity coefficient for the hydrogen ion. This was seldom found to be true, because of the impossibility of an exact standardization of the pH meter. Lack of such rigid accuracy in the standardization of the pH meter, however, did not influence the reliability of the determination, since the extrapolation was made to zero.

The apparatus used consists of a Beckman Model G glass electrode pH meter, a magnetic stirrer, air diffuser, 5-ml. microburet, graduated in 0.01 ml. with a platinum capillary tip, and a water bath maintained at 25° ± 1° C. The sample holder in some cases was a borosilicate glass beaker and in others a platinum dish. No significant loss in accuracy was noted when beakers were used at this temperature at pH less than 7. Stirring and air diffusion were continued for 2 minutes after the addition of each increment of acid and stopped during the period of pH determination.

Typical results on rain water samples are shown in Figure 2. The number for each extrapolation refers to different samples.

Numerically, alkalinity is defined as the equivalent concentration of titratable base and is determined by titration with a standard solution of a strong acid to certain equivalence points. In this procedure the equivalence point is taken to be at pH 7. The alkalinity is therefore equal to the equivalent concentration of titratable stronger base relative to a weaker acid, when using a standard solution of a strong acid. Therefore, ammonium acetate titrates fairly closely to an alkalinity equivalent of the ammonium ion concentration. Ammonium chloride has no alkalinity.

The acidity of a water is caused by carbon dioxide, mineral acids, and salts of strong acids and weak bases. Silicic, carbonic, and acetic acids are not as strong as mineral acids and therefore show no mineral acidity by this procedure. Acetic acid, however, can be titrated to a sufficiently low pH to expel carbon dioxide and back-titrated with excess 0.02*N* sodium hydroxide until complete ionization of acetic acid (99% at pH 7) is approached. Subsequent increments of sodium hydroxide then produce equivalent increases in OH^- activity. A straight-line extrapolation to 10^{-7} or to 0 mole (OH^-) per liter then indicates the concentration of weak acid. Care is essential to avoid reabsorption of carbon dioxide. Also, if ammonia is present it should be recognized that it becomes increasingly volatile with increase in pH, just as carbon dioxide is volatile at low pH.

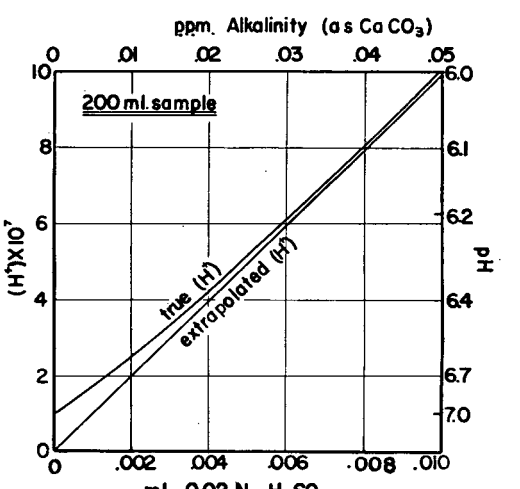


Figure 1. Calculated error introduced by extrapolation to zero instead of 1 × 10^{-7} mole (H^+) per liter for equivalence point

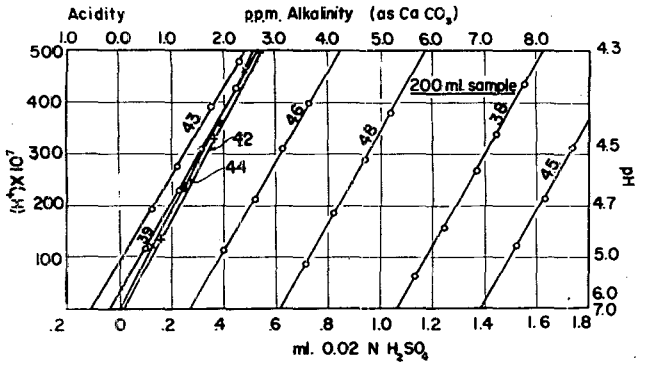


Figure 2. Extrapolations for typical rain water samples

Numbers (38 to 48) refer to laboratory sample numbers

Further consideration will show that both weak and strong acid concentrations in the same sample can be determined by titration first with 0.02*N* sulfuric acid and extrapolating to zero (H^+) for mineral acidity. This may then be followed by back-titrating with excess increments of 0.02*N* sodium hydroxide and extrapolating to zero (OH^-) per liter. The difference between the end points represents the weak acid acidity (exclusive of carbonic acid). The increasing solubility of glass at higher pH values limits the reliability of this application.

CONCLUSIONS

The procedure described for the determination of alkalinity has been found to be sensitive and accurate to ±0.05 p.p.m. alkalinity or mineral acidity when present in extremely low concentrations. A discussion has been provided on the titration of strong base–weak acid mixtures and the estimation of respective concentrations of strong acid and weak acid in acidic samples.

This procedure was devised for analysis of rain water. It is equally applicable for analysis of steam condensate and impurities in deionized water. Alkaline steam condensate samples should be collected with a known quantity of acid in order that ammonia is not volatilized before and during the titration. The procedure may also be adapted to analysis of air pollution samples.

It is limited to low concentrations and by the care and technique of the analyst. It can be made more sensitive and accurate by use of a more sensitive pH meter and a more accurate measurement of titrant volume.

LITERATURE CITED

(1) Am. Public Health Assoc., New York, "Standard Methods for the Examination of Water and Sewage," 9th ed., 1946.

RECEIVED for review September 14, 1954. Accepted December 29, 1954.

Correction Factors for Comparing Activities of Different Carbon-14–Labeled Compounds Assayed in Flow Proportional Counter

M. L. KARNOVSKY, J. M. FOSTER, L. I. GIDEZ, D. D. HAGERMAN, C. V. ROBINSON, A. K. SOLOMON, and C. A. VILLEE, *Biophysical Laboratory and Department of Biological Chemistry, Harvard Medical School, Boston, Mass.*

A comparison has been made of the activities of several organic compounds counted as such or as barium carbonate obtained after combustion. In the windowless gas-flow counter, under the conditions specified, the relevant correction factors are much smaller than those reported in the literature for end-window Geiger counters.

IN experiments with carbon-14 it is frequently necessary to compare the activities of different substances. For example, a comparison might be desired of the activity of a labeled substrate with the activity of carbon-14 dioxide derived from it metabolically or by chemical degradation. Correction factors have been reported to be necessary in making such conversions of the activities of organic substances (counted as such) to equivalent activities determined by counting the carbon-14 as barium carbonate. The following factors have, for example, been reported in experiments in which end-window Geiger Müller counters were used:

Glucosephenylosazone	1.34	(*5*)
Pyruvic-2,4-dinitrophenylhydrazone	1.26	(*1*)
Wax	1.29	(*8*)

Over the past several years, the authors have made similar measurements on a windowless gas-flow proportional counter. With this counting technique and using thin samples mounted on stainless steel planchets, correction factors are found under routine conditions of counting in this laboratory to be significantly lower than correction factors previously reported.

APPARATUS

The counter, used for these studies and described previously (*3*), is of the flow proportional type and uses a gas mixture of argon with 5% carbon dioxide. The dimensions are shown in Figure 1. The counter couples directly (without a cable) into the scaler-amplifier unit (Model 162, Nuclear Instrument and Chemical Corp., Chicago, Ill.) which is operated at about 1650 volts with a 5-mv. input sensitivity. The efficiency for thin carbon-14 samples is about 40%.

THEORETICAL

If a series of planchets of various thicknesses is made up from a single batch of material and counted, a curve obtained for counts *vs.* sample thickness is similar to that illustrated in Figure 2. This curve is characteristic of the sample material and can be used to make relative corrections between planchets of a given compound by correcting all counts to the same weight of sample.

To determine the ratio of activities between an organic compound and barium carbonate, the self-absorption curves must be known for each and the relative scales of the curves—i.e., the correction factor—must be determined. One way to do this is to burn the organic compound to obtain barium carbonate with the same activity per carbon.

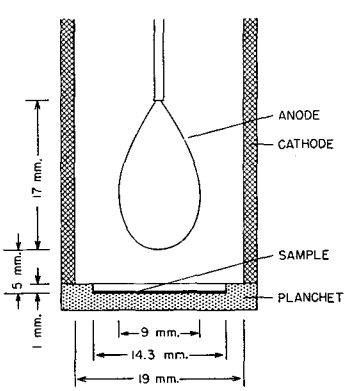


Figure 1. Windowless proportional flow counter

Self-absorption curves may then be obtained for the organic compound and for the barium carbonate derived from it. These curves may be related to each other in scale by adjusting the counts per minute in each case for the weight fraction of carbon in each compound.

Ratios of counts obtained from barium carbonate and those from the organic compound may be calculated from these curves for various

Table I. Specific Activity Counts per Minute per Millimole Carbon of Organic Substances and Barium Carbonate

Substance	Direct Count, Original Compd. (a)	Planchets counted	Direct Count, Derivative (b)	Planchets counted	Barium Carbonate from Original Compd. (c)	Planchets counted	Barium Carbonate from Derivative (d)	Planchets counted	Ratio, c/b	P^a
Glucose	...		3371 ± 118	20[b]	3424 ± 57	2	3373 ± 40	2	1.02 ± 0.04	0.70
Pyruvate	...		17131 ± 193	16[c]	16177 ± 342	8	...		0.94 ± 0.02	0.02
Glycerol										
1	...		199333 ± 4267	2[d]	209500 ± 1240	4	...		1.05 ± 0.03	<0.1
2	...		198167 ± 4080	4	213300 ± 3255	3	204500 ± 2450	6	1.08 ± 0.03	<0.15
Triolein										
1	897 ± 25	2	939 ± 12	3[d]	...		...		...	...
2	51600 ± 900	2	...		56400 ± 1455	6	...		1.09 ± 0.03[e]	<0.05

[a] Probability level for comparing columns from which ratio is calculated.
[b] Glucose phenylosazone.
[c] Pyruvic-2,4-dinitrophenylhydrazone.
[d] Formaldimedon.
[e] Ratio in this case is c/a.

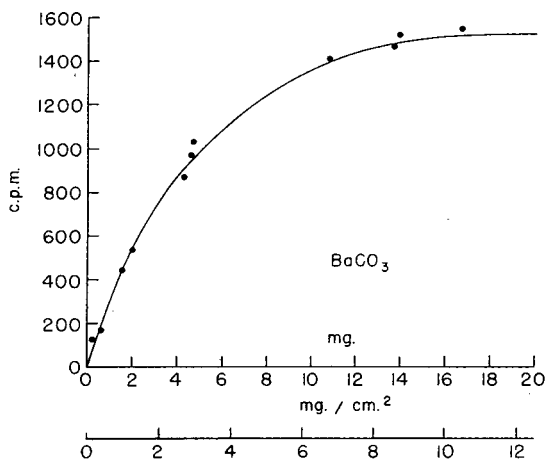


Figure 2. Self-absorption curve for barium carbonate

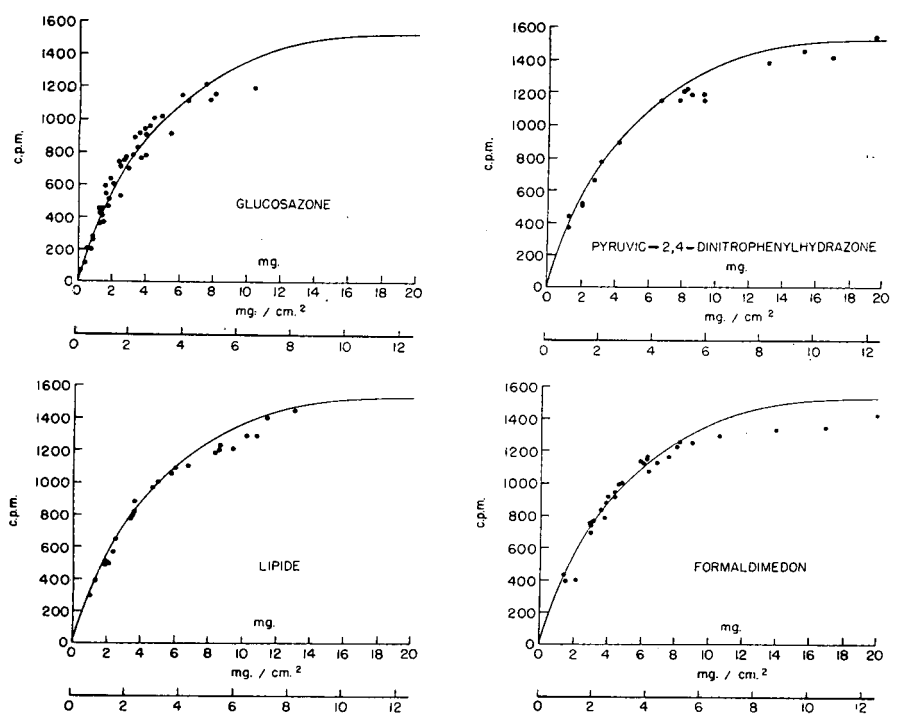


Figure 3. Self-absorption of various organic compounds

Solid line represents curve for barium carbonate; points are those determined for the different compounds. The lipide used was the total liver lipide of rats to which radioglycerol had been administered

weights and will be found to vary from unity for very thin samples to a maximum value characteristic of very thick samples (*2*, *8*). Backscattering is the important factor for thin samples and depends only on the backing material, whereas self-scattering, a function of the mean atomic number of the sample, becomes the important factor for thick samples (*2*).

The correction factors (*1*, *5*, *8*) are intermediate values of the ratios which apply to the working range of sample thicknesses in each instance with end-window Geiger-Müller counting of the particular geometry used.

EXPERIMENTAL

The barium carbonate self-absorption curve for the flow proportional counter was determined from a series of 15 planchets and checked by another series of 10. Each set was fitted by eye with a curve of the type $N = N_\infty(1 - e^{-\alpha m})$, where N is the number of counts at sample weight m, and N_∞ is the number of counts at infinite thickness. The average alpha is 0.322 sq. cm. per mg., which is in satisfactory agreement with the value obtained with end-window Geiger-Müller tubes (*4*). Figure 2 shows 11 points of the series of 15 points which fell within the weight range indicated, together with the theoretical curve normalized so that the 5-mg. point corresponding to 3.13 mg. per sq. cm., for a planchet area of 1.60 sq. cm., is 1000 counts per minute. The count for infinite thickness is then 1578 counts per minute.

Similar self-absorption curves were determined for each of four organic materials commonly encountered in metabolic studies: glucosazone, pyruvic-2,4-dinitrophenylhydrazone, lipide, and formaldimedon. The procedure is as follows: Beginning with a large amount of labeled material—for example, glucose—a derivative suitable for counting was made from part of it—for example, glucosazone—and another part was burned to make barium carbonate of the same activity per millimole of carbon.

For the labeled pyruvate, the derivative prepared was the 2,4-dinitrophenylhydrazone; that for α-labeled glycerol was formaldimedon. Triolein was synthesized from glycerol labeled as above and was counted as such; it was then saponified, the glycerol oxidized with periodate, and the resulting formaldehyde converted to formaldimedon. All derivatives were purified by thorough washing and recrystallization from dilute alcohol. Combustions of organic material to carbon dioxide and collection of barium carbonate for counting were carried out by the methods of Van Slyke and collaborators (*6*, *7*).

A series of planchets was made from each derivative and counted to give a self-absorption curve, and a number of barium carbonate planchets was made up from the carbon dioxide obtained by combustion in each case in order to determine the correction factor. As a

further check, carbon dioxide from combustion of the derivative was assayed as barium carbonate in some cases. The self-absorption curves for the four compounds are shown in Figure 3 and the combustion data in Table I.

RESULTS

The self-absorption curves for organic compounds were found to fit well with the barium carbonate curve by adjusting the scale. In Figure 3 the barium carbonate curve of Figure 2 is shown normalized to 1000 counts per minute at 5 mg. and the experimental points for the organic compounds are shown normalized in the same way. The fit is good from 1 to 7 mg. (0.06 to 4.4 mg. per sq. cm.) for all the compounds. Over the range mentioned the same self-absorption factors may be used for all the compounds studied.

The specific activity (counts per minute per millimole) of barium carbonate obtained from a given substance, adjusted for self-absorption to 5 mg. per planchet, has a value very near to that obtained (counts per minute per millimole carbon) from a derivative of the original substance similarly adjusted (Table I). The correction factors which are applicable to gas-flow counting under the conditions specified, near 5 mg. on the self-absorption curves, are considerably smaller than those previously reported for end-window Geiger-Müller tubes, and are, in fact, close to unity.

LITERATURE CITED

(1) Crane, R. K., and Ball, E. G., *J. Biol. Chem.*, **188**, 819 (1951).
(2) Glendenin, L. E., and Solomon, A. K., *Science*, **112**, 623 (1950).
(3) Robinson, C. V., *Ibid.*, **112**, 198 (1950).
(4) Solomon, A. K., Gould, R. G., and Anfinsen, C. B., *Phys. Rev.*, **72**, 1097 (1947).
(5) Topper, Y. J., and Hastings, A. B., *J. Biol. Chem.*, **179**, 1255 (1949).
(6) Van Slyke, D. D., and Folch, J., *Ibid.*, **136**, 509 (1940).
(7) Van Slyke, D. D., Steele, R., and Plazin, J., *Ibid.*, **192**, 769 (1951).
(8) Yankwich, P. E., and Weigl, J. W., *Science*, **107**, 651 (1948).

RECEIVED for review June 1, 1954. Accepted November 12, 1954. This investigation was supported by a contract with the U. S. Atomic Energy Commission, and by the Eugene Higgins Trust through Harvard University.

Qualitative Determination of Amino Acids in Protein Hydrolyzates by Circular Paper Chromatography

IRWIN ORESKES and ABRAHAM SAIFER

Department of Physical Chemistry, Division of Laboratories, Jewish Chronic Disease Hospital, Brooklyn 3, N. Y.

A circular chromatographic technique for the identification of amino acids in protein hydrolyzates uses phenol and butanol–acetic acid as the solvents, isatin and ninhydrin as the color reagents. R_f values compare favorably with those obtained by other investigators using buffered filter papers. The small size of apparatus and the speed and simplicity of the method make it very useful for routine analysis of amino acids. Quantitative applications of the technique are now under investigation.

IN A previous publication (*18*) studies of the physical factors that may influence circular paper chromatography in a closed system were discussed. The data obtained in this work supported the claims made by Rutter (*16, 17*) with respect to the many advantages of this simple chromatographic technique over the more conventional methods.

While the method has been criticized by some investigators (*13*) as being suitable for only simple amino acid systems, it has been shown by Giri and coworkers (*5, 6*) to be applicable to the complex amino acid mixtures found in protein hydrolyzates. In their latest publication dealing with the application of the circular chromatographic method to the determination of the 19 amino acids present in acid hydrolyzates of casein and edestin, Giri and Rao (*8*) employ a rather elaborate elution technique in conjunction with a four-solvent system. Such a system has little or no advantage over the multisolvent systems for the separation of complex amino acid mixtures—e.g., protein hydrolyzates—by one-dimensional paper sheet chromatography proposed by Fowden (*3, 4*), McFarren and Mills (*11*), and Redfield and Guzman Barron (*14*). More recently a unidimensional procedure suitable for the determination of the amino acid content of protein hydrolyzates has been published by Roland and Gross (*15*). These authors employ a two-solvent system for the separation of 16 out of the 19 amino acids usually found in such mixtures, but require two additional solvent systems with developing times of 40 and 64 hours, respectively, for the separation of tryptophan and histidine.

The circular chromatographic technique described in this paper will permit identification of all of the 19 amino acids usually found in protein hydrolyzates, except for leucines, which are determined as a pair. This is achieved by running two separate chromatograms for each hydrolyzate with phenol (0.1% ammonia) and butanol–acetic acid as the two developing solvents. After acetone washing to remove phenol and drying, each chromatogram is cut into halves, one half being dipped into ninhydrin and the other in isatin as the two coloring reagents. The usefulness of isatin as a color reagent that permits the rapid identification of a specific amino acid in a band containing several others has been described (*19*). This entire determination of the 19 amino acids can be carried out within 18 to 24 hours.

In addition to simplicity and compactness of apparatus, temperature control, speed and sharpness of separations, control of the rate of solvent flow, and removal of test samples during (or after) development, the circular chromatographic method described here has a high order of reproducibility of the R_f values for the individual amino acids, which compares favorably with the best of such values previously reported (*10*).

REAGENTS AND APPARATUS

Developing solvents were phenol, reagent grade (70%), reagent isopropyl alcohol (5%), and distilled water (25%) by weight (*20*).

A mixture of 1-butanol, acetic acid, and water (40–10–50% by volume), according to Partridge (*12*), is well shaken and the lower layer which forms is discarded.

Ammonia, 0.1%, was used in all phenol runs.

Amino acid solutions were made up according to Levy and Chung (*9*) in two solutions. Solution A contained 5 millimoles per liter each of leucine, phenylalanine, tryptophan, valine, proline, hydroxyproline, threonine, glycine, aspartic acid, and lysine in 0.1N hydrochloric acid. Solution B contained 5 millimoles per liter each of isoleucine, methionine, tyrosine, alanine, glutamic acid, serine, arginine, histidine, and cystine in 0.1N hydrochloric acid. The standard solutions were kept refrigerated when not in use. The solution to be chromatogramed was neutralized with an equal volume of 0.1N sodium hydroxide, just before application to the paper.

Color reagents used were isatin, 0.2% isatin in acetone containing 4% glacial acetic acid, and ninhydrin, 0.25% ninhydrin in acetone. These color reagents were applied to the chromatograms by dipping.

Whatman No. 1 filter paper, 24 cm. in diameter, was used in all runs.

Culture dishes and covers (*18*) were used for developing the chromatograms.

METHOD

The method of preparing and spotting the chromatograms as well as calculating the R_f values has been described (*18*). For each determination, two separate chromatograms were spotted with 20 μl. of neutralized standard solutions A and B.

One was developed with the phenol solution in the presence of 20 ml. of 0.1% ammonia solution placed in a 25-ml. beaker inside the culture dish. When the solvent front reached a radial distance of 8 cm., the chromatogram was removed and washed twice with anhydrous acetone (technical), which removed all traces of the phenol solvent. This procedure obviated the necessity of air drying and gave cleaner backgrounds and sharper bands.

The second chromatogram was developed with the butanol–acetic acid solution and upon completion allowed to air-dry. In this case washing with acetone was of no advantage, as the butanol evaporated quickly and had no deleterious effects upon the amino acids, as has phenol.

The completed chromatograms were then cut in half. One half was dipped in the isatin color reagent and the other half in the ninhydrin reagent. The sections were allowed to air-dry and develop color at room temperature for 3 to 4 hours, or, alternatively, they were heated for 10 minutes at 100° C. in order to hasten color development.

The culture dishes used in this work were found to be sufficiently air-tight for solvents such as phenol. In the case of butanol and other volatile solvents, evaporation could be prevented by placing the culture dishes inside ordinary plastic bags, which effectively prevented evaporation of solvent from the paper.

The hydrolyzate of human serum albumin was prepared by refluxing 125 mg. (0.5 ml.) with 25 ml. of 6*N* hydrochloric acid for 24 hours. (The 25% albumin solution was obtained from the American Red Cross through the courtesy of J. N. Ashworth. It was at least 95% pure by electrophoretic analysis.) The hydrochloric acid was removed by evaporation to dryness in vacuo at 35° C. (*1*). The resultant film of amino acids was taken up in 12.5 ml. of 0.1*N* hydrochloric acid, giving a concentration of 1.6 mg. of nitrogen per ml. As in the case of the standard solutions, an aliquot was neutralized with an equal volume of 0.1*N* sodium hydroxide just prior to use. Forty- and 80-μl. aliquots of the neutralized hydrolyzate were found suitable for spotting the chromatograms. The larger aliquot was necessary to detect methionine and other amino acids present in low concentrations.

RESULTS

Determination and Reproducibility of R_f Values. Twenty-four chromatograms containing the amino acids shown in Table I were run in the phenol solvent in the manner described above. The bands obtained were numbered beginning with the innermost one. The identities of the amino acids in each band were determined by chromatograming the standard mixture, to which an excess of one amino acid was added. Its position was then located by noting which band was darker or thicker. This procedure was used to locate the position of each amino acid, and thereby determine the order of separation shown in Table I.

Table I. Reproducibility of R_f Values Obtained in Standard Mixtures of Amino Acids Employing Phenol (0.1% Ammonia) as Developing Solvent

Band No.	Amino Acids Present	Mean R_f ± S.D.
1	Cystine	0.18 ± 0.023
2[a]	Aspartic acid	0.24 ± 0.020
3	Glutamic acid	0.35 ± 0.022
4	Serine	0.43 ± 0.024
5	Glycine	0.50 ± 0.021
6	Threonine	0.55 ± 0.022
7[b]	Alanine, tyrosine	0.64 ± 0.016
8	Hydroxyproline	0.71 ±0.010
9	Tryptophan	0.74 ± 0.019
10	Histidine, valine, methionine	0.80 ± 0.013
11	Leucines, lysine, phenylalanine, arginine	0.86 ± 0.011
12	Proline	0.92 ± 0.008

[a] Band sometimes splits into two bands.
[b] Occasionally this pair shows some separation.

Table II. Reproducibility of R_f Values Obtained in Standard Mixtures of Amino Acids Employing Butanol–Acetic Acid–Water (40:10:50) as Developing Solvent

Band No.	Amino Acids Present	Mean R_f ± S.D.
1	Cystine	0.18 ± 0.026
2[a] a	Lysine	0.22 ± 0.023
2[a] b	Histidine	0.23 ± 0.023
2[a] c	Arginine	0.25 ± 0.023
3	Aspartic acid, glycine, serine	0.31 ± 0.018
4	Hydroxyproline	0.33 ± 0.022
5	Glutamic acid, threonine	0.36 ± 0.018
6	Alanine	0.40 ± 0.018
7	Proline	0.44 ± 0.026
8[b] a	Tyrosine	0.45 ± 0.021
8[b] b	Tryptophan	0.47 ± 0.021
9	Methionine	0.55 ± 0.020
10[b] a	Valine	0.62 ± 0.016
10[b] b	Phenylalanine	0.66 ± 0.016
11	Leucines	0.73 ± 0.014

[a] These three amino acids travel close together.
[b] At high concentrations these two amino acids tend to overlap.

An identical series of 24 runs was performed using butanol–acetic acid as the developing solvent (Table II).

The mean R_f of a component for a single run is obtained as the average of six different measurements (*18*). The average R_f values and their standard deviation for the 24 determinations are given for phenol in Table I and for butanol in Table II.

Qualitative Identification of Amino Acids in Protein Hydrolyzates. The scheme for the qualitative identification of the amino acids in a protein hydrolyzate as developed in this laboratory employs two circular chromatograms run simultaneously. One is developed in phenol and the other in the butanol solvent, the former being washed in acetone after development. Half of each chromatogram is then dipped in isatin and the other half in ninhydrin. If the standard mixtures are assumed to represent a hypothetical protein hydrolyzate containing all 19 amino acids, their identification could then be made in the following manner:

On the phenol-ninhydrin chromatogram the following amino acids separate as pure bands and can be easily identified: cystine, aspartic acid (blue-green), glutamic acid, serine, glycine, threonine, and tryptophan (brown). Hydroxyproline and proline are identifiable as pure bands on the phenol-isatin portion. In this system alanine and tyrosine migrate together, but as only tyrosine reacts with isatin, it is also identifiable on the isatin portion.

On the butanol-ninhydrin chromatogram, cystine, arginine, alanine, methionine, and the leucines separate as pure bands. However, valine and phenylalanine tend to overlap at high concentrations. The identification is made on the butanol-isatin portion, where only the outer part of the band reacts, showing the presence of phenylalanine. Valine is identified by a positive ninhydrin reaction and a negative isatin reaction of the inner part of the band. With approximately 10 γ of valine and phenylalanine, resolution usually occurs which permits their direct identification. Lysine and histidine travel close together in butanol and their identification is sometimes difficult with ninhydrin. However, in isatin the lysine gives a light lavender shade and the histidine a dark blue-gray color, thus making their identification possible. Except where otherwise noted, the ninhydrin colors are shades of purple and the isatin colors are blue-green.

In a number of cases isatin allows identifications to be made even where two or more components migrate close together or overlap on the chromatogram. This eliminates the necessity for complete resolution of all the components. As it is possible to cut out several sectors from circular chromatograms, many other color reactions can be used to confirm the presence of specific amino acids (*1, 7, 20*) on a single chromatogram.

The application of this technique to the acid hydrolyzate of human serum albumin is shown in Figures 1 and 2. Except for the presence of alanine, the amino acids found in this hydrolyzate were the same as those reported by other investigators (*2, 21*).

DISCUSSION

As stated by McFarren (*10*), conventional, two-dimensional paper sheet chromatography suffers from the difficulties of poorly defined spots, irreproducible R_f values, inseparability of some of the amino acids, and the manipulation of large paper sheets. This makes one-dimensional chromatography inherently more suitable for the quantitative analysis of the amino acids present in protein hydrolyzates. It was essentially for this reason that a number of investigators (*3, 4, 8, 10, 11, 14, 15*) have employed unidimensional paper chromatography using multisolvent developing systems.

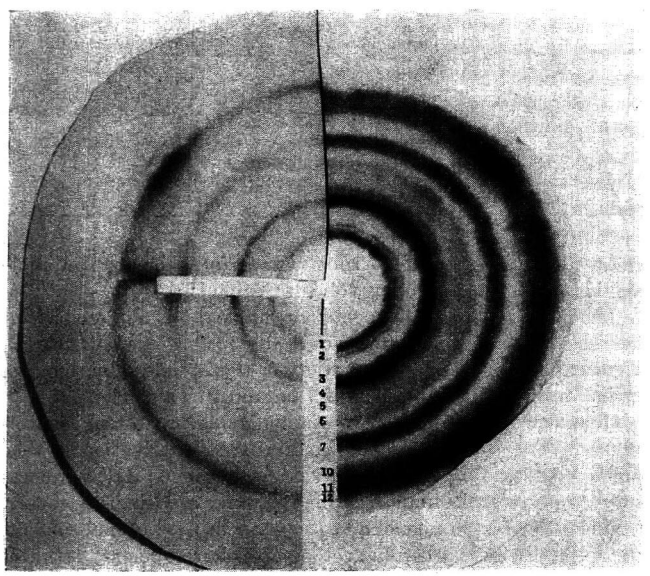


Figure 1. Circular chromatogram of hydrolyzate of human serum albumin developed with phenol

Left, colored with isatin; right, colored with ninhydrin. Sequence corresponds to amino acids listed in Table I. Chromatogram used to identify the following amino acids: (1) cystine, (2) aspartic acid, (3) glutamic acid, (4) serine, (5) glycine, (6) threonine, (7) tyrosine, (12) proline. Hydroxyproline and tryptophan were not present

Circular chromatography has one major advantage over other chromatographic systems in which the amino acids are resolved as spots: the separation of the components as narrow bands (1 to 4 mm. in width), so that substances can be identified, even though their R_f values differ by only 0.01 to 0.02 unit. This makes possible the separation and identification of such groups as lysine-histidine-arginine and valine-phenylalanine, which travel close together in this system.

In general, R_f values reported in the literature show great variability due to variations in pH, temperature, and filter paper used. McFarren (*10*) in an extensive study on the separation of amino acids using buffered filter papers reported R_f values and their standard deviation at a number of pH's and in a variety of solvents. Using phenol as the solvent, and by controlling pH and temperature (22° ± 1° C.), he obtained standard deviations of the amino acid R_f values ranging from ±0.01 to ±0.05. The present work was done with unbuffered papers and at room temperature, and without close temperature control. Even so, the largest standard deviation was ±0.026 for cystine and proline. This indicates that with the circular chromatographic method, R_f values can be obtained with excellent reproducibility, if the factors previously investigated are carefully controlled (*18*). The reproducibility is as good as or better than that obtained with buffered papers and the extra step of preparing the buffered papers is not required. However, where the amino acids travel closely together—e.g., basic amino acids—the identification should be based primarily on sequence and color rather than on the R_f values.

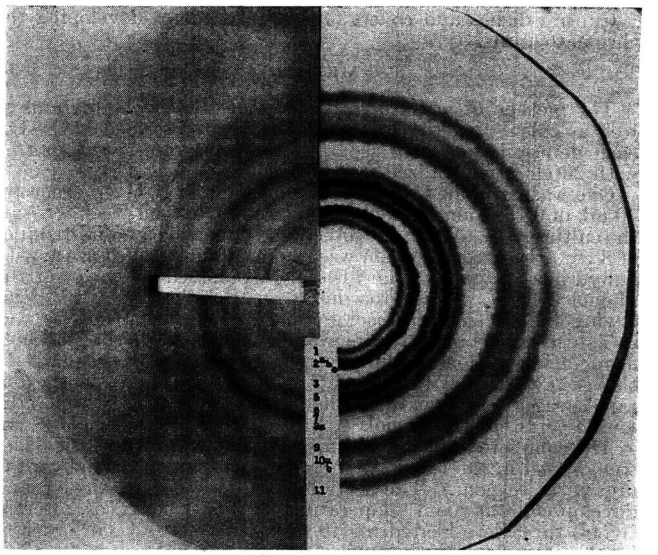


Figure 2. Circular chromatogram of hydrolyzate of human serum albumin developed with butanol–acetic acid

Left, colored with isatin; right, colored with ninhydrin. Sequence corresponds to amino acids listed in Table II. Chromatogram used to identify the following amino acids: (1) cystine, (2a) lysine, (2b) histidine, (2c) arginine, (6) alanine, (7) proline, (8a) tyrosine, (9) methionine, (10a) valine, (10b) phenylalanine, (11) leucines. Hydroxyproline and tryptophan were not present. Because of color differences, separations of 2a, 2b, and 2c are more apparent on the actual chromatogram than on the photographic reproduction

The quantitative determination of the amino acids present in various biological materials, the instrumentation involved, and the techniques employed will be discussed in a subsequent publication.

ACKNOWLEDGMENT

The authors wish to acknowledge the aid and encouragement given them by Bruno W. Volk, director of laboratories, throughout this study, and to express appreciation to Renee Eisner for the typing and editing of the manuscript and to Herbert Fischler for the photographic illustrations.

LITERATURE CITED

(1) Block, R. J., ANAL. CHEM., **22**, 1327 (1950).
(2) Brand, E., *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946).
(3) Fowden, L., *Biochem. J. (London)*, **48**, 327 (1951).
(4) *Ibid.*, **50**, 355 (1952).
(5) Giri, K. V., *Nature*, **171**, 1159 (1953).
(6) Giri, K. V., Krishnamurthy, K., and Venkitasubramanyan, T. A., *J. Indian Inst. Sci.*, **34**, 209 (1952).
(7) Giri, K. V., and Nagabhushanam, A., *Naturwissenschaften*, **39**, 548 (1952).
(8) Giri, K. V., and Rao, N. A. N., *J. Indian Inst. Sci.*, **35**, 343 (1953).
(9) Levy, A. L., and Chung, D., ANAL. CHEM., **25**, 396 (1953).
(10) McFarren, E. F., *Ibid.*, **23**, 168 (1951).
(11) McFarren, E. F., and Mills, J. A., *Ibid.*, **24**, 650 (1952).
(12) Partridge, S. M., *Biochem. J. (London)*, **42**, 238 (1947).
(13) Proom, H., and Woiwood, O. J., *Nature*, **171**, 42 (1953).
(14) Redfield, R. R., and Guzman Barron, E. S., *Arch. Biochem. and Biophys.*, **35**, 443 (1952).
(15) Roland, J. F., Jr., and Gross, A. M., ANAL. CHEM., **26**, 502 (1954).
(16) Rutter, L., *Analyst*, **75**, 37 (1950).
(17) Rutter, L., *Nature*, **161**, 435 (1948).
(18) Saifer, A., and Oreskes, I., ANAL. CHEM., **25**, 1539 (1953).
(19) Saifer, A., and Oreskes, I., *Science*, **119**, 124 (1954).
(20) Toennies, G., and Kolb, J. J., ANAL. CHEM., **23**, 823 (1951).
(21) Tristram, G. R., *Advances in Protein Chem.*, **5**, 83 (1949).

RECEIVED for review April 29, 1954. Accepted November 12, 1954. Presented before the Division of Biological Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y., 1954. Aided by Grant B-285 from the U. S. Public Health Service.

Quantitative Determination of Glucose and Galactose

HANS FISHER[1], R. G. HANSEN, and HORACE W. NORTON

University of Illinois, Urbana, Ill.

A rapid and sensitive method, based upon the orcinol procedure of Brückner, is used for the simultaneous estimation of glucose and galactose in mixtures. Results are usually within 0.03 mg. for glucose and 0.01 mg. for galactose in the concentration range of 0 to 0.150 mg. of each sugar. Two systems for evaluating the data are presented.

THE simultaneous quantitative determination of glucose and galactose in biological materials is of importance in the study of galactose and lactose metabolism in various animals. Galactosemia in infants (*1, 12, 25*) and the etiology of Gaucher's disease, where a glucosidic cerebroside is found in addition to the normal galactose derivative (*7, 14, 17–19, 21*) are specific examples of application of such a procedure.

A variety of techniques has been used for qualitative or approximately quantitative determination of these two sugars. Fermentation by selective yeasts has been extensively used (*15, 17, 18, 24*). The removal of glucose by glucose oxidase has also been employed (*23*) for the determination of galactose in blood plasma. A number of chromogenic tests have been applied to this problem: carbazole reactions (*8, 9, 13*), *o*-tolylhydrazine (*11*), sulfuric acid–orcinol (*2–5*), and reducing sugar equivalents of the two hexoses obtained by the methods of Folin-Wu and Sumner (*7, 10*). Finally, paper chromatographic procedures have also been recommended (*1, 6, 23*).

For quantitative estimation of these two sugars, most of the above procedures lack specificity or are too time-consuming for ordinary application. In some preliminary work the orcinol procedure of Brückner seemed to offer the greatest promise of quantitative adaptation. This report records a modification of Brückner's method which permits rapid simultaneous estimation of glucose and galactose.

Table I. Absorbance of Glucose and Galactose

Glucose, Mg.	Galactose, Mg.	Absorbance, Replicate 1, 470 mμ	Absorbance, Replicate 1, 560 mμ	Absorbance, Replicate 2, 470 mμ	Absorbance, Replicate 2, 560 mμ
0	0.050	0.130	0.248	0.120	0.240
0	0.100	0.278	0.500	0.255	0.480
0	0.150	0.373	0.685	0.405	0.740
0	0.200	0.530	0.925	0.525	0.925
0.050	0	0.055	0.065	0.052	0.058
0.100	0	0.123	0.137	0.110	0.128
0.150	0	0.178	0.198	0.178	0.192
0.200	0	0.250	0.264	0.250	0.264
0.050	0.050	0.180	0.303	0.188	0.310
0.050	0.100	0.328	0.541	0.313	0.547
0.050	0.150	0.455	0.770	0.450	0.765
0.100	0.050	0.238	0.360	0.268	0.410
0.150	0.050	0.322	0.443	0.302	0.428
0.100	0.100	0.412	0.640	0.378	0.610

In Brückner's method concentrated sulfuric acid is added to the sample containing orcinol, the heat of solution providing the necessary conditions for color development. As the development of color is rapid and greatly influenced by temperature, adequate mixing and rapid chilling 8 to 20 seconds after mixing are important. More prolonged heating periods increase color density but reduce initial differences in the absorption spectra of the two sugars. That the authors were unable to achieve good reproducibility by the suggested procedure was undoubtedly due to slight differences in mixing or length of time before chilling.

In order to overcome these objections, all reagents were added to the spectrophotometer tube maintained in an ice bath. Immediately after addition of the sulfuric acid the solution is thoroughly mixed with the aid of a capillary stream of nitrogen. Under these conditions no appreciable color develops without subsequent heating, which can then be best accomplished in a constant temperature bath for a fixed time.

[1] Present address, Poultry Department, Rutgers University, New Brunswick, N. J.

SOLUTIONS

Sulfuric acid, $3N$.

Sulfuric acid, 92% (94 ml. of concentrated sulfuric acid, specific gravity 1.84, and 6 ml. of water).

Orcinol, 2 grams of orcinol in 50 ml. of water, to which is added a cooled solution made by mixing 20 ml. of concentrated sulfuric acid and 30 ml. of water.

Standard glucose and galactose solutions containing 0.1 mg. per ml. in $3N$ sulfuric acid.

PROCEDURE

An aliquot of the sample containing up to 0.2 mg. of total sugar is placed in a spectrophotometer tube and adjusted to a volume of 2 ml. with water and enough sulfuric acid to bring the solution to $3N$. Two milliliters of orcinol solution are next added while the tubes are cooled in an ice bath. Then 6 ml. of the sulfuric acid reagent are rapidly added and mixing is completed with a stream of nitrogen through a capillary. The capillary is rinsed in $3N$ sulfuric acid between immersions. The tubes are then heated exactly 4 minutes in a bath maintained at 67–68° C. with good circulation. After 5 minutes' cooling in an ice bath, color density is estimated at 470 and 560 mμ.

STANDARD CURVES

A replicated series of tubes containing glucose and galactose individually and in the various combinations in concentrations ranging from 0 to 0.2 mg. per tube was treated as outlined in the procedure. Absorption curves for the individual glucose and galactose solutions are shown in Figure 1. Absorbance readings at 470 and 560 mμ appear to permit the greatest sensitivity of measurement of concentration of the two sugars. The absorbance measurements for a standard series at the specified wave lengths are given in Table I. This standard series was one of several determined at various times. These series were in good mutual agreement.

CALCULATION OF RESULTS

Planes passing through the origin fitted by least squares are

$$\hat{u} = 1.22x + 2.63y$$
$$\hat{v} = 1.35x + 4.73y$$

which can be solved for x and y, giving

$$\hat{x} = 2.13u - 1.18v$$
$$\hat{y} = -0.61u + 0.55v$$

where x and y are, respectively, the milligrams of glucose and galactose in the spectrophotometer aliquot, and $\hat{u}$ and $\hat{v}$ are the estimated absorbances at 470 and 560 mμ.

Statistical analysis (Table II) showed that quadratic surfaces passing through the origin represented the data more satisfactorily, the fitted functions being

$$\hat{u} = 1.05x + 2.57y + 0.98x^2 + 1.32xy + 0.32y^2$$
$$\hat{v} = 1.27x + 5.08y + 0.21x^2 - 0.06xy - 2.27y^2$$

The glucose and galactose values given in Table III are calculated from these equations. A sample calculation and the confidence limits of the values obtained are given in the discussion of statistical analysis.

The error introduced by using the simpler equations never exceeds 0.03 mg., in either x or y, in the region of the standard data, in which the sum of x and y (both positive) does not exceed

Table II. Multivariate Analysis of Data Given in Table I

Source	Degrees of Freedom	Sum of Squares for u	Sum of Products	Sum of Squares for v
Quadratic surface	5	2.570681	4.270116	6.998016
Deviations	9	0.000426	0.000523	0.000827
Error	14	0.002313	0.002624	0.003695

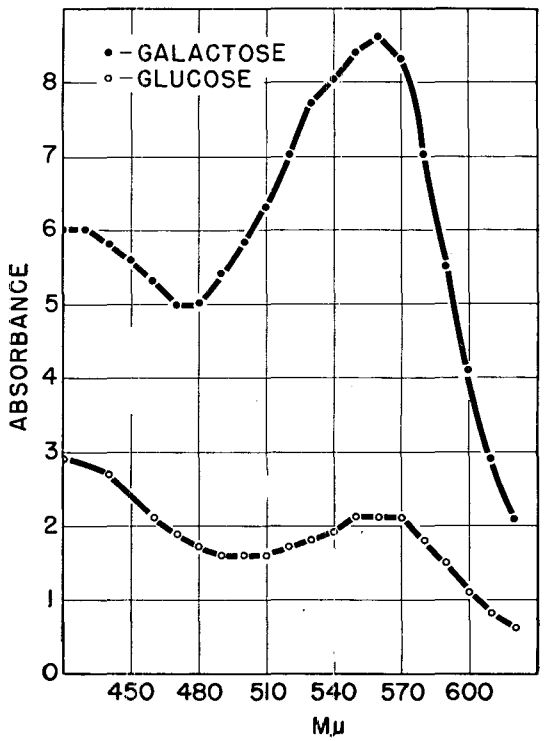


Figure 1. Absorbance of glucose and galactose measured in Coleman Junior spectrophotometer

0.2 mg. This may suggest that they would often be sufficiently accurate, but errors of 0.01 mg. may be much larger than the experimental errors and so might entirely obscure what would otherwise be easily detected as a statistically significant difference.

APPLICATION OF METHOD TO BLOOD FILTRATES

By feeding chicks purified diets containing varying amounts of glucose, galactose, lactose, and corn meal, blood that contained mixtures of sugars was available for study. The plasma was deproteinized with zinc and barium hydroxide (Somogyi) and aliquots of the filtrate were taken for sugar determination. Total reducing substance was estimated by a modified Somogyi procedure, in which 0.5 ml. of butanol was layered above the mixture during boiling and the boiling period was extended to 30 minutes.

Separate aliquots of the plasma filtrate were also deionized with a mixture of IR 45 and Dowex 50 (about 10 to 1). They were then concentrated by lyophilization and the aqueous solution was spotted on chromatograms. An ethyl acetate–pyridine-water solvent system (*16*) was used to develop the chromatograms and aniline oxalate (*20*) was used to locate the spots.

The partitioned glucose and galactose values for the blood by the orcinol determination are given in Table III, together with the total reducing values by Somogyi's method. The agreement obtained points to the accuracy and usefulness of the method. The reproductions of the chromatograms shown in Figure 2 further validate these results.

DISCUSSION

The nature of the absorption curves for glucose and galactose as shown in Figure 1 serves as an indicator of the usefulness and limitations of the present method. Thus, ribose has an absorption band similar to that of glucose, so that this absorption appears to be relatively nonspecific. On the other hand, galactose has a very specific spectrum and its determination at 470 and 560 mμ is extremely sensitive to small changes in the concentration of a substance exhibiting a glucoselike absorption spectrum.

In applying the orcinol method to brain extracts for cerebroside sugar determinations, it was found that pentose-containing compounds are present in appreciable quantities which must be extracted. As shown by chromatography, galactose was the only sugar present in pentose-free extracts of chicken brain. The orcinol method applied to the same extracts gave small values for glucose besides the expected galactose values, illustrating that other substances besides pentoses absorb in the glucose region. This is not surprising, as the concentrated sulfuric acid employed will produce a slightly yellow color with all organic materials which, depending on their relative concentration with respect to galactose, can influence the glucose values obtained. When applied to pure phrenosine, however, the present method indicated only galactose, as expected.

STATISTICAL ANALYSIS

The standard data occur in pairs, an absorbance at 470 mμ and at 560 mμ, each pair measured on a single sample. The experimental errors affecting the two data are probably correlated, and multivariate analysis (*22*) is appropriate. It might be hoped that the data could be represented by two planes passing through the origin, but a priori considerations suggest that curvature is likely to become apparent at the higher absorbances. This was

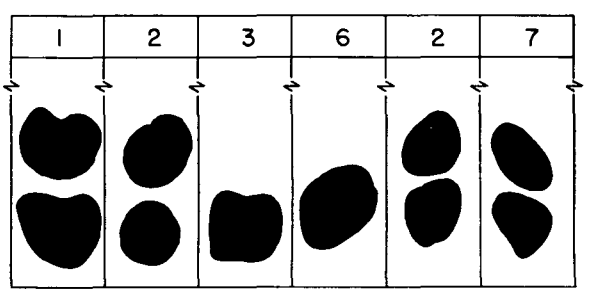


Figure 2. Reproductions of chromatograms of blood filtrates

1. **Glucose + galactose diet**
2. **Standard galactose (upper spot) and glucose (lower spot)**
3. **Glucose diet**
6. **Glucose + lactose diet**
7. **Corn + galactose diet**

Table III. Determination of Glucose and Galactose in Blood

	Orcinol Method				Somogyi Method	
CHO in Diet	470 mμ	560 mμ	Glucose[a]	Galactose[a]	515 mμ	Reducing sugar[a]
Glucose	0.14	0.16	0.116	0.002	0.50	0.098
	0.11	0.13	0.097	0.002	0.44	0.086
Corn meal	0.16	0.16	0.151	−0.007	0.51	0.100
	0.15	0.16	0.134	−0.003	0.49	0.096
Glucose + 30% lactose	0.14	0.17	0.106	0.007	0.50	0.098
	0.14	0.17	0.106	0.007	0.47	0.092
Glucose + 15% galactose	0.44	0.70	0.108	0.116	0.80	0.197[b]
	0.37	0.62	0.063	0.111	0.65	0.170[b]
Corn meal + 15% galactose	0.33	0.52	0.098	0.080	0.70	0.167[b]
Std. glucose					0.51	0.100
Std. galactose					0.31	0.100

[a] Milligrams/0.05 ml. of chicken blood.
[b] Calculated on basis of ratio of glucose to galactose found by orcinol method.

clearly shown by the data, though the curvature is not great. The multivariate analysis of the standard data appears in Table II.

Quadratic surfaces passing through the origin were fitted, so that five constants had to be evaluated from the data. Hence the analysis shows 5 degrees of freedom for the sums of squares and products attributable to regression, the remaining 9 (among 14 locations) for deviations from regression, and 14 degrees of freedom for experimental error, arising from differences between duplicate determinations at each location. Comparison of the covariance matrix for deviations to that for error shows that the quadratic surfaces fit the data satisfactorily, and the deviation and error matrices may be pooled to provide estimates of error based on 23 degrees of freedom.

These data provide an interesting example of the effectiveness of multivariate analysis. It was found that two planes through the origin did not fit satisfactorily. However, judged separately, each passed as near to the origin as it should if the true surface were a plane through the origin. Only when the two planes were tested simultaneously did it appear that the fit was inadequate. In fact, the intercept for one plane was found to be positive and for the other negative, incompatible with the very high correlation in the experimental errors. This was discernible only by multivariate analysis.

The fitted quadratic surfaces can be used as indicated above to estimate the glucose and galactose concentrations in an unknown. Furthermore, the precision of the estimates can be expressed in terms of the several derivatives and the precision of the standard data. To a first approximation,

$$\mu(x^2) = M^2[D^2\mu(u^2) - 2BD\mu(uv) + B^2\mu(v^2)]$$
$$\mu(xy) = M^2[-CD\,\mu(u^2) + (AD + BC)\,\mu(uv) - AB\,\mu(v^2)]$$
$$\mu(y^2) = M^2[C^2\,\mu(u^2) - 2AC\,\mu(uv) + A^2\,\mu(v^2)]$$

where $\mu(x^2)$ is the variance (mean squared error) of the estimate of x, and $\mu(xy)$ is the covariance of the estimates of x and y, and A, B, C, D, and M are defined below. The variances and covariance of u and v come from the error line of Table II, or better from pooling deviations and error. This gives $\mu(u^2) = 0.000119$, $\mu(uv) = 0.000137$, $\mu(v^2) = 0.000197$, on 23 degrees of freedom. The approximate variances and covariance of the estimates of x and y will be satisfactory approximations if M is several times as large as its sampling error. For the standard data used here, M exceeds 100 times its sampling error.

Given observed values of u and v for an unknown, the quadratic equations

$$\hat{u} = 1.05x + 2.57y + 0.98x^2 + 1.32xy + 0.32y^2$$
$$\hat{v} = 1.27x + 5.08y + 0.21x^2 - 0.06xy - 2.27y^2$$

can be solved for x and y. This is most easily done arithmetically. Letting $\frac{du}{dx} = A$, $\frac{du}{dy} = B$, $\frac{dv}{dx} = C$, $\frac{dv}{dy} = D$, and $AD - BC = 1/M$, corrections to assumed values of x and y are

$$\Delta x = M(D\Delta u - B\Delta v)$$
$$\Delta y = M(A\Delta v - C\Delta u)$$

where

$$\Delta u = u - \hat{u}$$
$$\Delta v = v - \hat{v}$$

This process starts conveniently at $x = y = 0 = \hat{u} = \hat{v}$, and needs no more than two repetitions to arrive at estimates of x and y which are substantially as precise as the data can furnish.

For an example of the calculations, take $u = 0.140$, $v = 0.160$ (line 1 of Table III). Starting from $x = y = 0 = \hat{u} = \hat{v}$, the first values of A, B, C, D, and M are, respectively, 1.05, 2.57, 1.27, 5.08, and 0.483, and Δu and Δv are 0.140 and 0.160. Hence $\Delta x = 0.145$ and $\Delta y = 0.005$. Taking $x = 0.145$, $y = 0.005$, $\hat{u} = 0.187$, $\hat{v} = 0.214$, $A = 1.34$, $B = 2.76$, $C = 1.33$, $D = 5.05$, $M = 0.323$, and $\Delta u = -0.047$, $\Delta v = -0.054$. Hence $\Delta x = -0.029$, $\Delta y = -0.003$, and $x = 0.116$, $y = 0.002$. These values of x and y give $u = 0.1404$ and $v = 0.1603$, so no further improvement is needed. The new values of A, B, C, D, and M are 1.28, 2.72, 1.32, 5.06, and 0.346. These may be used to calculate the variances and covariance of $\hat{x}$ and $\hat{y}$. In particular, the variance of $\hat{x}$ is given by $\mu(x^2) = [(5.06)^2(0.000119) - 2(2.72)(5.06)(0.000137) + (2.72)^2(0.000197)](0.346)^2$, which comes to 0.0000878. Taking the square root, the sampling error of $\hat{x}$ is found to be 0.0094. Multiplying by 2.069, the 5% value of t for 23 degrees of freedom, we find that values within 0.019 of $\hat{x}$ are acceptable at the 5% level—that is, the 95% confidence interval is $0.097 \leq x \leq 0.135$.

The variance of y is 0.00000804, so that y is estimated over ten times as well as x. The covariance is −0.00001215, and may be used to establish confidence limits for x and y simultaneously, or for functions of x and y. For example, limits on the sum or the difference of x and y might be needed.

The size of the experimental error may prove to be much affected by interferences. In that event, the error of estimating x and y can be reduced by inferring them from values of u and v which are the averages of replicate determinations. Finally, the extremely high correlation between experimental errors at the two wave lengths suggests that the method is capable of further improvement.

CONCLUSIONS

The procedure as modified is sensitive and reproducible for glucose as the only sugar in biological materials and eliminates standards to be run with each determination. It is extremely sensitive and reproducible for galactose as the only sugar in purified materials (phrenosine), but suffers from interference of sugars other than glucose present in crude brain extracts. It is most useful in the quantitative determination of both glucose and galactose, particularly in blood but probably also in other materials. Statistical analysis shows that results should nearly always be within 0.03 mg. for glucose and 0.01 mg. for galactose in the concentration range specified.

ACKNOWLEDGMENT

H. E. Carter, Chemistry Department, University of Illinois, kindly supplied the phrenosine. Financial support was received from the U. S. Atomic Energy Commission contract No. AT (11-1) - 67, project No. 10.

LITERATURE CITED

(1) Bickel, H., and Hickmans, E. M., *Arch. Disease Childhood*, **27**, 348 (1952).
(2) Brückner, J., *Z. physiol. Chem.*, **268**, 163 (1941).
(3) *Ibid.*, p. 251.
(4) *Ibid.*, **275**, 73 (1942).
(5) *Ibid.*, **377**, 181 (1943).
(6) Chargaff, E., Levine, C., and Green, C., *J. Biol. Chem.*, **175**, 67 (1948).
(7) Danielson, I. S., Hall, C. H., and Everett, M. R., *Proc. Soc. Exptl. Biol. Med.*, **49**, 569 (1942).
(8) Dische, Z., Shettles, L. B., and Osnos, M., *Arch. Biochem.*, **22**, 169 (1949).
(9) Edman, P. V., *J. Biol. Chem.*, **143**, 219 (1942).
(10) Everett, M. R., and Edwards, B. G., *Ibid.*, **100**, xlii (1933).
(11) Fowweather, F. S., *Biochem. J.*, **55**, 718 (1953).
(12) Goldbloom, A., and Brickman, H. F., *J. Pediat.*, **28**, 674 (1946).
(13) Gurin, S., and Hood, D. B., *J. Biol. Chem.*, **131**, 211 (1939).
(14) Halliday, N., Deuel, H. J., Jr., Tragerman, L. J., and Ward, W. E., *Ibid.*, **132**, 171 (1940).
(15) Harding, V. J., and Grant, G. A., *Ibid.*, **94**, 529 (1931).
(16) Jermyn, M. A., and Isherwood, F. A., *Biochem. J.*, **44**, 402 (1949).
(17) Klenk, E., *Z. physiol. Chem.*, **267**, 128 (1940).
(18) Lieb, H., and Gunther, V., *Ibid.*, **271**, 211 (1941).
(19) Ottenstein, B., Schmidt, G., and Thannhauser, S. J., *Blood*, **3**, 1250 (1948).
(20) Partridge, S. M., Biochemical Society Symposia, No. 3, Cambridge University Press, London, 1950.
(21) Polonovski, J., *Bull. soc. chim. biol.*, **25**, 44 (1943).
(22) Rao, C. R., "Advanced Statistical Methods in Biometric Research," Wiley, New York, 1952.
(23) Rutter, W. J., Krichevsky, P., Scott, H. M., and Hansen, R. G., *Poultry Sci.*, **32**, 706 (1953).
(24) Sie, Hsien-Giels, Faust, C. E., and Williams, H. H., *Federation Proc.*, **7**, 161 (1948).
(25) Townsend, E. H., Jr., Mason, H. H., and Strong, P. S., *Pediatrics*, **7**, 760 (1951).

RECEIVED for review April 19, 1954. Accepted December 10, 1954.

Determination of Soluble Ortho-, Pyro-, and Triphosphate in Presence of Each Other

L. E. NETHERTON, A. R. WREATH, and D. N. BERNHART

Victor Chemical Works, Research Laboratories, Chicago Heights, Ill.

A rapid, simple, and accurate procedure for the analysis of mixtures containing water-soluble ortho-, pyro-, and triphosphates has been developed based upon the selective alkaline hydrolysis of triphosphate combined with a modified colorimetric method for determining orthophosphate.

IT HAS been shown that sodium triphosphate hydrolyzes slowly in 1% sodium hydroxide solution at 100° C. to form equimolar quantities of orthophosphate and pyrophosphate (*1*). Under these conditions, approximately 60 hours were required for complete hydrolysis.

This same reaction takes place in 40 to 60 minutes when the triphosphate is heated at the boiling temperature with a 10 to 20% sodium hydroxide solution. By contrast, sodium pyrophosphate is stable toward hydrolysis under the prescribed conditions.

These observations have served as the basis for a new and superior method for the determination of sodium triphosphate by measuring colorimetrically the amount of orthophosphate developed following alkaline hydrolysis. The phosphorus content determined as orthophosphate following hydrolysis, multipled by three, represents the phosphorus content due to the triphosphate originally present. The hydrolysis of sodium triphosphate in hot concentrated sodium hydroxide solution may be represented by Equation 1.

$$Na_5P_3O_{10} + 2NaOH \longrightarrow Na_4P_2O_7 + Na_3PO_4 + H_2O \qquad (1)$$

Most commercial samples of sodium triphosphate contain approximately 10% tetrasodium pyrophosphate and zero to a few tenths of 1% of sodium orthophosphate. In a review of methods for determining sodium triphosphate and tetrasodium pyrophosphate in the presence of each other, Dewald and coworkers (*4*) concluded that no accurate method exists for triphosphate samples containing between 4 and 10% of pyrophosphate. A simple, rapid, and accurate method for analyzing such products is described by the authors and has been applied to the analysis of commercial sodium triphosphate and to synthetic mixtures containing varying percentages of ortho-, pyro-, and triphosphates.

Table I. Analysis of Phosphates

	P_2O_5, %		Bell Method[a]	
	Calcd.	Found	Pyro, %	Tri, %
$Na_4P_2O_7$	53.4	53.4	99.8	...
$Na_5P_3O_{10}$	57.9	57.8	Nil	100.0
KH_2PO_4	52.2	52.1	..	...

[a] Results (*2*) are for sodium triphosphate hexahydrate and not anhydrous sodium triphosphate.

The following procedure is recommended.

Orthophosphate. The orthophosphate content of the mixture is determined colorimetrically prior to alkaline hydrolysis.

Triphosphate. A sample of the mixture is hydrolyzed in 10 to 20% sodium hydroxide solution at the boiling temperature for 40 to 60 minutes, diluted to a known volume, and orthophosphate colorimetrically determined. The difference in orthophosphate content before and after hydrolysis is calculated to triphosphate.

Pyrophosphate. The mixture is hydrolyzed completely in hot acid solution, cooled, and total orthophosphate determined colorimetrically. The total phosphorus content, minus that due to triphosphate and orthophosphate present originally, is equivalent to the phosphorus content of the pyrophosphate in the original sample.

Since most samples of commercial sodium triphosphate contain very little, if any, orthophosphate or metaphosphate, and since pyrophosphate is unaffected under the prescribed conditions, the estimation of the sodium triphosphate content can be made by a colorimetric orthophosphate determination, following alkaline hydrolysis of the sample. All other chain and cyclic phosphates will interfere.

EXPERIMENTAL

Apparatus. A Klett-Summerson photoelectric colorimeter was used at 430 mμ with a 10-mm. test tube for the colorimetric determination of orthophosphate.

Reagents. PHOSPHATES. The tetrasodium pyrophosphate (*2*) was prepared from the commercial grade product by three recrystallizations and by heating the resulting hydrated tetrasodium pyrophosphate at 400° C. to convert it to the anhydrous form. The sodium triphosphate was prepared by recrystallization of high purity (approximately 98%) commercial sodium triphosphate, using ethyl alcohol as the precipitant. The sodium triphosphate hexahydrate (*2*) was dehydrated by heating at 380° to 400° C. for 20 hours. Reagent grade potassium dihydrogen phosphate was used without further purification. Results for these reagents are summarized in Table I.

AMMONIUM MOLYBDATE SOLUTION. This solution was prepared by dissolving 18.75 grams of ammonium molybdate, reagent grade, in 300 ml. of water, adding carefully 150 ml. of concentrated sulfuric acid, cooling, and diluting to 500 ml.

ACETONE, C.P.

SODIUM HYDROXIDE (50% solution). One thousand grams of C.P. sodium hydroxide were dissolved in 1000 ml. of water. The solution was filtered through an asbestos pad and stored in a plastic bottle.

STANDARD PHOSPHATE SOLUTION. Potassium dihydrogen phosphate, reagent grade (0.9578 gram) which had been dried for 1 hour at 110° C. was dissolved in a small amount of water and diluted to 500 ml. One milliliter of this solution contains 1 mg. of phosphorus pentoxide.

Procedure. A 1-gram sample was dissolved in water and diluted to 250 ml. Using an appropriate aliquot, the original orthophosphate (*3*) content of the sample was determined. On another aliquot of the same size the total phosphorus content (*3*) of the sample was determined following acid hydrolysis. To a third aliquot (25 ml.) in a 250-ml. beaker, 25 ml. of 50% sodium hydroxide solution and 100 ml. of water were added. The solution was covered with a watch glass and placed on a hot plate. After the sample had been boiled for 45 minutes and had been concentrated to about 50 to 80 ml., it was removed from the hot plate, and cooled to room temperature, then transferred to a 100-ml. volumetric flask and diluted to volume. A 10-ml. aliquot was then transferred to a 50-ml. flask in an ice bath. In the meantime, a mixture containing 100 ml. of the ammonium molybdate solution, 150 ml. of acetone, and 150 ml. of water was made up and chilled in the ice bath. The sample was diluted to 50 ml. with this acetone–ammonium molybdate–water solution, mixed well, and the absorbance determined after 1 minute.

The phosphorus content of the orthophosphate present at this stage, minus the phosphorus content of the orthophosphate originally present in the sample, multiplied by three represents the phosphorus content of the sodium triphosphate in the original sample. Tetrasodium pyrophosphate is determined by difference. The total phosphorus content of the sample minus that due to triphosphate and orthophosphate represents the phosphorus content of the pyrophosphate in the original sample.

Hydrolysis of Sodium Triphosphate to Pyrophosphate and Orthophosphate. Aliquots containing 0.1000 gram of sodium triphosphate or tetrasodium pyrophosphate were boiled in 150 ml. of 5, 10, 20, and 30% sodium hydroxide solution. Solution samples were removed at time intervals of 15, 30, 45, 60, and 120 minutes, cooled, and diluted to 100 ml. The solutions had concentrated approximately to one third to one half of their original volume during the boiling. No attempt was made to keep them at constant volume.

Then 10-ml. aliquots (equivalent to 10 mg. of the original sample) were analyzed for orthophosphate using a modified colorimetric method (*3*). The data in Table II show that after 45 minutes in 10% sodium hydroxide solution or 30 minutes in 20% and 30% sodium hydroxide solution, one mole of orthophosphate was formed per mole of sodium triphosphate originally present, as required by Equation 1. The tetrasodium pyrophosphate did not hydrolyze to orthophosphate under any of the conditions as shown in Table II. It was therefore established that the hydrolysis of sodium triphosphate in hot alkaline solutions proceeds according to Equation 1.

Table II. Hydrolysis of Sodium Triphosphate and Pyrophosphate in Boiling Sodium Hydroxide Solutions

Original Concn. of NaOH, %	Moles of Ortho Formed					
	$Na_5P_3O_{10}$					$Na_4P_2O_7$
	15 min.	30 min.	45 min.	60 min.	120 min.	120 min.
5	0.25	0.40	0.65	0.78	0.90	0.0
10	0.75	0.95	1.00	1.00	1.00	0.0
20	0.85	1.00	1.00	1.00	1.00	0.0
30	0.95	1.00	1.00	1.00	1.00	0.0

Modified Colorimetric Method. For determining orthophosphate in presence of pyrophosphate and sodium hydroxide, the modified colorimetric method of Bernhart and Wreath (*3*) employing a sulfuric acid solution of ammonium molybdate in an acetone-water medium was used. A standard curve for phosphorus pentoxide content versus absorbence, ranging from 0 to 3 mg. of phosphorus pentoxide, was prepared using a Klett-Summerson photoelectric colorimeter at 430 mμ with a 10-mm. test tube. No changes in the values of this standard curve were found when the standard phosphate solution (0, 1, 2, and 3 mg. of phosphorus pentoxide) contained 10 mg. of tetrasodium pyrophosphate and 2.5 ml. of 50% sodium hydroxide solution and the absorbence was read within 30 minutes.

Table III. Determination of $Na_5P_3O_{10}$ in Mixtures of $Na_4P_2O_7$ and $Na_5P_3O_{10}$

Triphosphate Added[a], %	$Na_5P_3O_{10}$ Found, %	Difference	Deviation, Parts per Thousand
100	100.0	0.0	0
	99.8	−0.2	2
	99.7	−0.3	3
	99.3	−0.7	7
95	94.8	−0.2	2
	94.4	−0.6	6
	94.5	−0.5	5
	94.5	−0.5	5
90	90.6	+0.6	7
	90.3	+0.3	3
	90.3	+0.3	3
	90.2	+0.2	2
85	85.5	+0.5	6
	85.0	0.0	0
	84.1	−0.9	11
	85.0	0.0	0
80	80.5	+0.5	6
	79.3	−0.7	9
	80.4	+0.4	5
60	59.6	−0.4	7
	59.7	−0.3	5
	59.6	−0.4	7
40	39.8	−0.2	5
	40.0	0.0	0
	40.2	+0.2	5

[a] Remainder is $Na_4P_2O_7$.

During the investigation of the effect of tetrasodium pyrophosphate and sodium hydroxide on the standard phosphate curve, certain changes had to be made in the original colorimetric method. When the ammonium molybdate reagent was added first, the heat evolved due to neutralization of the base was sufficient to cause rapid hydrolysis of the pyrophosphate in the acid solution, yielding high results. Cooling in an ice bath did not entirely eliminate hydrolysis of pyrophosphate to orthophosphate. When the ammonium molybdate reagent was added last an off-color developed. Increasing the acetone concentration or decreasing the sulfuric acid concentration caused a separation into two liquid layers. Excellent results were obtained when the acetone, water, and ammonium molybdate reagent were mixed, cooled in an ice bath, and added to the chilled aliquot containing the phosphates and sodium hydroxide. The volume ratio of the acetone, water, and ammonium molybdate solutions was 1.5 to 1.5 to 1.0. No hydrolysis of the pyrophosphate occurred when this procedure was followed.

RESULTS

The results obtained on analyzing triphosphate and pyrophosphate mixtures are given in Table III and demonstrate the accuracy and reproducibility of this method of analysis.

Although data are presented only for mixtures containing 40% or more of sodium triphosphate and 60% or less of tetrasodium pyrophosphate, the method is also applicable for mixtures richer in tetrasodium pyrophosphate. The average time required for an individual analysis amounts to approximately 70 minutes. The time may be drastically reduced in the analysis of a series of samples by hydrolyzing all of the samples simultaneously.

Synthetic mixtures of orthophosphate, pyrophosphate, and triphosphate were prepared from the purified reagents and analyzed by this procedure. The results as presented in Table IV show that the new method gives satisfactory results for these and similar types of mixtures.

Table IV. Analysis of Ortho-, Pyro-, and Triphosphate Mixtures

Composition %	% Found	Difference
Ortho 5.0	5.0, 5.0	0.0, 0.0
Pyro 5.0	4.4, 4.3	−0.6, −0.7
Tri 90.0	90.3, 90.5	+0.3, +0.5
Ortho 5.0	5.0, 5.0	0.0, 0.0
Pyro 10.0	9.1, 9.3	−0.9, −0.7
Tri 85.0	85.5, 85.2	+0.5, +0.2
Ortho 5.0	5.0, 5.0	0.0, 0.0
Pyro 15.0	14.2, 14.0	−0.8, −1.0
Tri 80.0	80.4, 80.8	+0.4, +0.8
Ortho 5.0	5.0, 5.0	0.0, 0.0
Pyro 20.0	19.0, 19.4	−1.0, −0.6
Tri 75.0	75.6, 75.2	+0.6, +0.2

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Esther Paciorek, who performed most of the analyses reported, and the helpful criticism of L. F. Audrieth and R. N. Bell during the preparation of the manuscript.

LITERATURE CITED

(1) Bell, R. N., *Ind. Eng. Chem.*, 39, 136 (1947).
(2) Bell, R. N., Wreath, A. R., and Curless, W. T., ANAL. CHEM., 24, 1997 (1952).
(3) Bernhart, D. N., and Wreath, A. R., *Ibid.*, 27, 440 (1955).
(4) Dewald, W., Schmidt, M., and Neeb, K. H., *Fette u. Seifen*, 56, 105 (1954).

RECEIVED for review June 28, 1954. Accepted November 11, 1954. Presented before the Division of Analytical Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York.

Identification of Organic Bases by Means of the Optical Properties of Dilitrates (Nitrobarbiturates)

Secondary Aromatic Amines

BARTLETT T. DEWEY[1], *Eastern New Mexico University, Portales, N. M.*, and
ELMER M. PLEIN, *University of Washington, Seattle, Wash.*

THE optical and crystallographic properties of some primary and secondary aliphatic amines and primary aromatic amines have already been reported by the authors (*1–3*). The present paper gives similar data for some secondary aromatic amine dilitrates.

The dilitrates were prepared by mixing a hot aqueous solution of dilituric acid with an equivalent quantity of the liquid amine or with a hydrochloric acid or acetic acid solution of the solid amine. The dilitrates were recrystallized from water. The purity of the compounds was established by an analysis by the Kjeldahl method modified to include nitro compounds.

Attempts to prepare the dilitrates of some amines not soluble in hydrochloric acid by using acid alcohol or acid Cellosolve as solvents failed to yield a product which contained the calculated amount of nitrogen.

The crystallographic and optical properties were determined by the same procedures used in the previous works. Table I gives the crystal system, the maximum extinction angle, the optic sign, the refractive indices, the elongation, and the dispersion. Table II shows the apparent properties of the crystals from their orientation in the immersion liquids. Ethylaniline dilitrate and *p*-methylaminophenol dilitrate orient themselves in a position which presents a centered obtuse bisectrix figure. The values for alpha and beta can be determined from this orientation. Methylaniline dilitrate, methyl-*m*-toluidine dilitrate, and *n*-propylaniline dilitrate present a centered optic normal figure. Thus alpha and gamma may be observed on these crystals in their usual orientation. The dilitrates of isoamylaniline, benzylaniline, *n*-butylaniline, ethyl-*m*-toluidine, ethyl-*p*-toluidine, and *β*-hydroxyethylaniline assume a position which gives a true value for beta on the majority of crystals. Ethyl-*o*-toluidine dilitrate usually presents a true value for alpha. When differences in orientation occur, so that no consistent value for the refractive indices is apparent on most crystals, the values are designated as variable. The optical properties must be determined on freshly

[1] Deceased.

Table I. Optical Properties of Some Secondary Aromatic Amine Dilitrates

Dilitrate	System	Extinction Angle	Optic Sign	Refractive Indices			Elongation	Dispersion
				Alpha	Beta	Gamma		
n-Amylaniline	T	32	−	1.503	1.604	1.623	±	$\nu > \rho$
Isoamylaniline	M	41	−	1.453	1.651	1.680	±	$\nu > \rho$
p-Benzylaminophenol	M	29	−	1.489	1.659	>1.785	+	$\nu > \rho$
Benzylaniline	T	40	−	1.524	1.714	1.736	±	$\nu > \rho$
n-Butylaniline	M	40	−	1.508	1.656	1.664	−	$\rho > \nu$
Ethylaniline	O	0	−	1.563	1.663	1.676	−	$\nu > \rho$
Ethyl-1-naphthylamine	M	4	−	1.590	1.703	>1.785	+	$\rho > \nu$
Ethyl-*o*-toluidine	M	11	−	1.442	1.697	1.749	−	$\nu > \rho$
Ethyl-*m*-toluidine	M	32	−	1.540	1.655	1.702	−	$\nu > \rho$
Ethyl-*p*-toluidine	M	26	−	1.498	1.675	1.696	−	$\nu > \rho$
β-Hydroxyethylaniline	M	8	−	1.458	1.696	1.767	±	$\nu > \rho$
p-Methylaminophenol	M	44	−	1.627	1.719	1.762	±	$\nu > \rho$
Methylaniline	M	40	−	1.616	1.724	1.737	+	$\rho > \nu$
Methyl-1-naphthylamine	M	36	+	1.575	1.603	>1.785	±	$\rho > \nu$
Methyl-*o*-toluidine	M	23	−	1.480	1.718	1.779	−	$\nu > \rho$
Methyl-*m*-toluidine	M	39	−	1.637	1.675	1.709	−	$\rho > \nu$
Methyl-*p*-toluidine	T	39	−	1.535	1.698	>1.785	±	$\nu > \rho$
m-Nitromethylaniline	M	25	+	1.520	1.612	>1.785	±	$\rho > \nu$
n-Propylaniline	O	0	−	1.572	1.613	1.649	−	$\nu > \rho$

Table II. Apparent Properties of Secondary Aromatic Amine Dilitrates from Most Frequently Observed Orientation

Dilitrate	Habit	Optical Orientation	Extinction Angle	Refractive Indices	
n-Amylaniline	Equant	Inclined optic axis	Variable	Variable	Variable
Isoamylaniline	Tabular	Inclined optic axis	0	Variable	1.651
p-Benzylaminophenol	Equant	Inclined acute	Variable	Variable	Variable
Benzylaniline	Tabular	Inclined optic axis	Variable	Variable	1.714
n-Butylaniline	Tabular	Inclined optic axis	Variable	Variable	1.656
Ethylaniline	Lath	Obtuse	0	1.563	1.663
Ethyl-1-naphthylamine	Lamellar	Inclined acute	0	1.703	>1.785
Ethyl-*o*-toluidine	Lath	Obtuse	11	1.442	Variable
Ethyl-*m*-toluidine	Lath	Inclined obtuse	0	Variable	1.655
Ethyl-*p*-toluidine	Lamellar	Inclined obtuse	0	Variable	1.675
β-Hydroxyethylaniline	Lath	Variable obtuse	0	Variable	1.696
p-Methylaminophenol	Tabular	Obtuse	Variable	1.627	1.719
Methylaniline	Tabular	Optic normal	40	1.616	1.737
Methyl-1-naphthylamine	Columnar	Variable	0	1.603	Variable
Methyl-*o*-toluidine	Tabular	Inclined obtuse	0	Variable	Variable
Methyl-*m*-toluidine	Tabular	Optic normal	39	1.637	1.709
Methyl-*p*-toluidine	Tabular	Inclined optic axis	Variable	Variable	Variable
m-Nitromethylaniline	Tabular	Inclined obtuse	0	Variable	>1.785
n-Propylaniline	Tabular	Optic normal	0	1.572	1.649

recrystallized material. Many of the diliturates readily lose water of crystallization, with a resulting change in the optical properties.

In the figures are diagrams of the crystals, showing front views as the crystals appear in their most frequently occurring orientation and side views and top views obtained by rolling the crystals in Canada balsam. Dotted lines indicate vibration directions, and apparent refractive indices are recorded for crystals which show constant values. An asterisk indicates the higher value on views where no consistent values could be obtained. Crystal angles measured microscopically are indicated at the corners of the diagrams.

LITERATURE CITED

(1) Dewey, B. T., and Plein, E. M., IND. ENG. CHEM., ANAL. ED., **18**, 515 (1946).
(2) Plein, E. M., and Dewey, B. T., ANAL. CHEM., **27**, 307 (1955).
(3) Plein, E. M., and Dewey, B. T., IND. ENG. CHEM., ANAL. ED., **15**, 534 (1943).

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

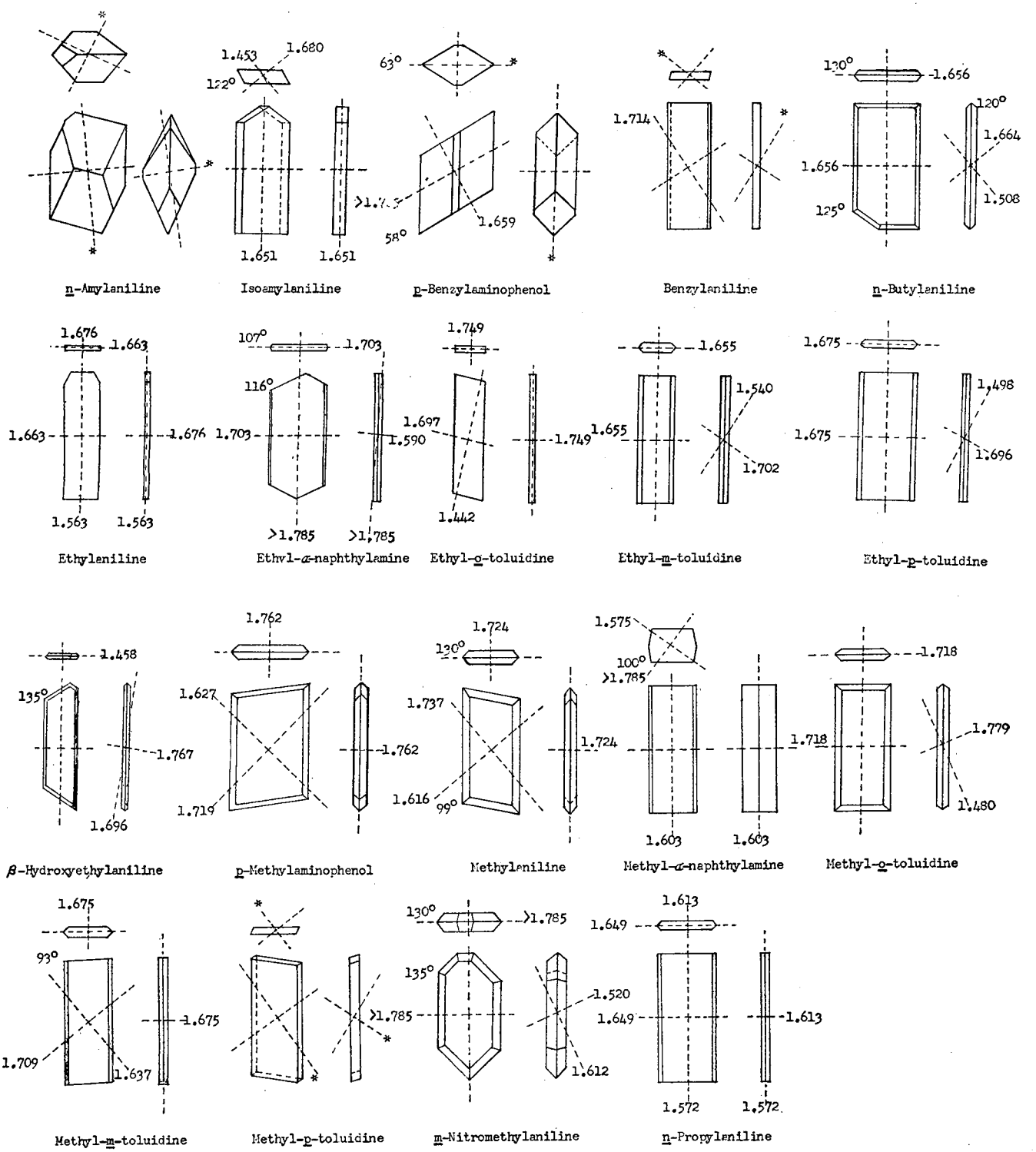


Figure 1. Diagrams of crystals of amine diliturates

Microchemical Symposium

THE Tenth Annual Microchemical Symposium, sponsored by the Metropolitan Microchemical Society, on the theme of "Micromethods for Determining Physical Constants," was held March 18 and 19 at the American Museum of Natural History, New York, N. Y.

Petroleum Hydrocarbons. FREDERICK D. ROSSINI, Petroleum Research Laboratory, Carnegie Institute of Technology, Pittsburgh, Pa.

The work of the American Petroleum Institute Research Project 6 on the analysis, purification, and properties of petroleum hydrocarbons, now in its 28th year of operation, is to learn what petroleum consists of in terms of hydrocarbon compounds. To do this, the project has had to develop and operate fractionating processes to a degree of separating power much greater than is necessary in ordinary research operations. To identify the hydrocarbon compounds isolated from petroleum in this way, the project requires highly purified compounds for comparison. To provide these reference samples for research, and at the same time to provide samples to the industry for spectrometric analyses, the project prepares highly purified hydrocarbons of the API Research series for fundamental measurements and of the API Standard series for industrial research, testing, analysis, and control. On the highly purified API Research hydrocarbons, the project makes accurate measurements of freezing points (including determination of purity), densities, refractive indices, and boiling points and vapor pressures. An account of this work was given from the standpoint of its applicability to the theme of the symposium.

New Approach to Molecular Weights by Use of a Dynastat. J. K. OWENS, Instrument Specialties, West Chester, Pa.

A new osmometric type of approach to the determination of number average molecular weight of materials was presented, in which the precision of the static method is retained and combined with the speed of the dynamic method. The method covers in part the range normally not attainable by osmometric means—1000 to 20,000. The total range at present is 600 to 1,500,000. A distinct advantage is the fact that mixed solvents can be used successfully on such materials as polyamides, polyesters, polypeptides, and sucrose. The determination can be run in less than 1 hour after solution is effected and solutions of 4% concentration have been tested.

Cytochemical Micrurgy. M. J. KOPAC, Department of Biology, Graduate School of Arts and Science, New York University, New York, N. Y.

The evaluation of physicochemical constants at levels consistent with the dimensions of living cells represents one of the most difficult problems in contemporary experimental biology. Two micrurgical approaches, at present, are feasible. With one, the distribution of enzymes in living cells may be determined; with the other, information on absolute kinetics of certain enzymes in the living cell may be obtained. The only information now available concerning the kinetics of enzyme-modulated reactions is derived from enzymes no longer associated with living systems and, in most instances, the integration between enzymes that normally exists in the living cell has been lost during isolation.

Several recent developments have given reliable information concerning the distribution of enzymes in relation to the various cytoplasmic and nuclear structures. For the hydrolytic enzymes, we have the microdilatometric method which is capable of measuring activities of the order of micromicromoles per hour. Associated with the microdilatometer are the necessary instruments for preparing enzyme-substrate reaction mixtures at micro levels and for obtaining samples of protoplasm at submicro levels. One of these instruments, the volumetric submicromanipulator, is capable of removing known volumes of cellular substance as small as micromicroliters.

Another example of instrumentation is the development of the ultraviolet micromanipulator. By application of the television principle, the invisible ultraviolet image is picked up by a Vidicon camera and instantly translated into a visible image on the television screen. Since nucleoproteins, by virtue of the purine and pyrimidine bases contained in the nucleotides, are opaque to ultraviolet light at 260 mμ, we have a convenient means of studying the action of extrinsic nucleases, applied by microinjection, on cellular structures rich in nucleic acids.

Another feature, combining ultraviolet micrurgy with microdilatometry, permits an attack on the absolute kinetics of RN-ases and DN-ases in the cell.

Numerous problems of theoretical and practical importance are now being studied. One of the unsolved problems is to characterize the so-called cancer cell. Attempts have been made to compare the enzymology of cancer cells with those of their normal ancestors. The results have been discouraging because, frequently, the total enzyme content is not significantly different between normal and cancer cells. On the other hand, the distribution of enzymes within the cells may be strikingly different and such differences may be determined only by suitable fractionation and measurements. These procedures were described.

Ebulliometry. JOHN R. ANDERSON, Mellon Institute of Industrial Research, Pittsburgh, Pa.

Ebulliometry is defined as the technique of precise measurement of boiling and condensation temperatures of solutions. Determination of condensation temperatures poses no particular problem. The ways that have been contrived for measuring boiling temperatures, their limitations and shortcomings, and the uses and misuses that have been made of the data were described. Special attention was given to ebulliometric determination of molecular weight and of purity.

Physical Constants in Organic Analysis. FRANK SCHNEIDER, Department of Chemistry, Queens College, Flushing, N. Y.

Among the objectives of the Metropolitan Microchemical Society was its intention to serve as a medium for establishing certain standards, not only for critical pieces of equipment, but also for tests and data. In working with tables of physical constants in qualitative organic analysis, the author found such variation in the values of these constants as to render them, in some cases, entirely useless as a means of identification. In seeking the cause of such variation, it was found that, while different methods were used for the determination, no clarifying notation is included in the tables. Obviously, a standard method of reporting values, or, better, a standard method of determining values should be adopted. The selection of a standard method requires careful study of the sources of error and the means of minimizing or eliminating them. An example of such a critical examination of a method of a physical constant determination is described in the present paper. Standardization of determination and reporting of physical constant data is recommended and certain forms are proposed.

Physical Constants with the Microscope. CHARLES MARESH, Research Division, American Cyanamid Co., Bound Brook, N. J.

The microscopical methods have long been synonymous with micromethods for the determination of physical constants. Although not experiencing the growth of other instrumental methods in the past decade, the polarizing microscope is gradually being accepted by the chemist. Optical and crystallographic constants, of prime importance in identification and analysis, are used to advantage as an adjunct to structure determination by x-ray diffraction as well as in explaining the physical behavior of many of our natural and man-made products. In more recent years the methods developed by the Koflers in Germany have provided a new approach to the collecting of physical constants.

Characterization of Hydrated Aluminas—Correction

In the article on "Characterization of Hydrated Aluminas by Infrared Spectroscopy" [Frederickson, L. D., Jr., ANAL. CHEM., 26, 1883 (1954)] reference (15) should read: Fichter, R., *Helv. Chim. Acta*, 30, 2010 (1947).

Unfortunately, this article was not included in the subject index for 1954. Entries should have appeared under the headings "infrared," "alumina," "spectrometry," "spectra," "spectrophotometry," "diaspore," and "bauxite."

Automatic Cut-Off Valve for Ion Exchange Columns

Clifford A. Hewitt, National Bureau of Standards, Washington 25, D. C.

In the operation of ion exchange or chromatographic columns, the bed of the column must always be covered with liquid. If the level of the liquid falls below the top of the bed, the separation being made is usually vitiated because of the introduction of air into the bed. Such air in the column bed is difficult to remove and tends to cause channeling.

To maintain the liquid level above the bed level of the column, many investigators have extended the exit tube to above the bed level of the column. This tube prevents air from being drawn into the bed (*1–3*).

A very simple device, easily constructed, can prevent the level of the liquid from falling below the top of the column bed, and, in so doing, permit the simultaneous operation of many similarly equipped columns without fear of failure. Furthermore, the device permits any definite amount of liquid to enter the column.

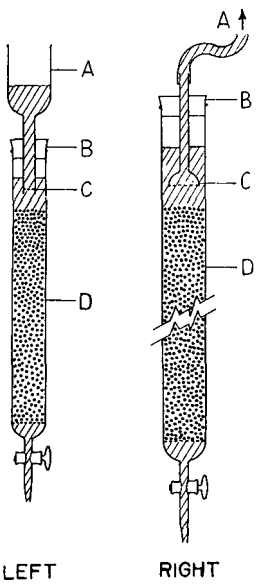


Figure 1. Arrangement of safety valve

Left. For analytical work
Right. For usual ion exchange column

A. Reservoir
B. Rubber stopper
C. Fritted disk
D. Resin bed

The device or valve consists of an immersion tube provided with a frit, the fritted end being immersed in the liquid at the top of the column. The stem of the tube passes through a rubber stopper which tightly fits the glass column. In operation the level of the solution above the resin should be about half way between the bottom of the rubber stopper and that of the fritted disk. The flow of liquid through the disk stops when the level of the liquid added reaches that of the fritted disk. At this state the level of liquid in the column proper may drop slightly.

The fritted disk prevents the liquid in the column from draining because the surface tension of the solution in the pores of the frit is sufficient to prevent air from passing through. Thus a partial vacuum is created above the liquid in the column. When this equals the hydrostatic head, drainage stops. Because it is immersed in the liquid, the disk serves to reduce turbulence when the column is in operation. The disk does not serve to regulate the rate of flow through the column.

In analytical separations, where a definite volume of liquid is known to strip the column of a certain element, the entire volume of the eluting solution can be added at one time; automatic cutoffs will thus enable the operator to manage a considerable number of columns.

Figure 1, left, shows the arrangement for an analytical column and right that for the usual ion exchange column where *A* is the reservoir, *B* is the rubber stopper, *C* is the fritted disk, and *D* is the resin bed. In the column for analytical separations, it is necessary to use a small frit sealed to the stem of the reservoir tube so that the tube can be inserted through the rubber stopper.

A frit of medium porosity would maintain a column of water of about 250 cm. in height, and one of fine porosity a column of about 450 cm. The immersion tubes are commercially available with fritted glass disks of 10, 30, and 60 mm. in diameter. For practical purposes the medium frit functions well. The fritted tubes also serve to filter the eluting solutions, but may gradually become clogged; however, they can be cleaned with some suitable reagent, as, for instance, nitric acid.

LITERATURE CITED

(1) Samuelson, Olof, "Ion Exchangers in Analytical Chemistry," pp. 79–82, Wiley, New York, 1953.
(2) Tompkins, E. R., *J. Chem. Educ.*, **26**, 92 (1949).
(3) Tompkins, E. R., Khym, J. X., and Cohn, W. E., *J. Am. Chem. Soc.*, **69**, 2769 (1947).

Plastic Dry Box

Andrew J. Franklin and Sterling E. Voltz,
Houdry Process Corp., Marcus Hook, Pa.

A simple device has been designed for use in procedures which require the handling of chemicals under "dry box" conditions. A satisfactory plastic dry box constructed out of readily available materials has been successfully employed in procedures which require the transfer of materials under extremely dry conditions, such as the transfer of samples pretreated in a high vacuum system, the preparation of samples for Karl Fischer titrations, and the preparation of infrared samples.

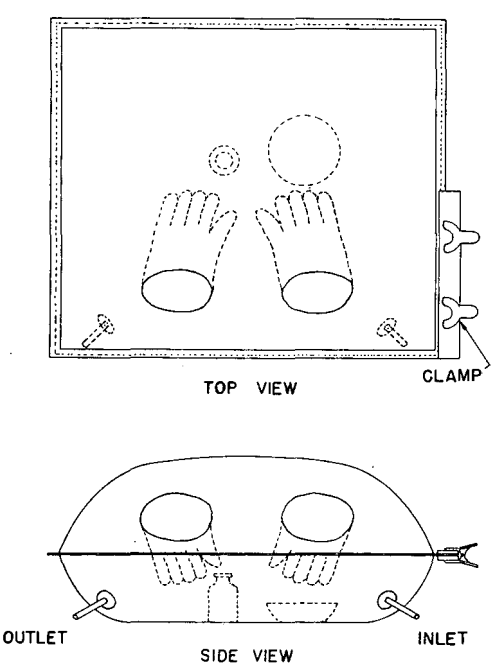


Figure 1. Plastic dry box

One plastic dry box is illustrated in Figure 1. The most satisfactory plastic materials were those which are sold commercially for use in plastic storm windows. These materials are completely transparent and have a relatively low permeability to water vapor. The plastic was cut to the desired shape and sewed together to form a plastic bag as shown in the figure. The moistureproof seals were then made by pressing a hot flat iron on the edges (including about 1/4 inch on the inside of the stitching) for several seconds. A pair of plastic gloves was constructed out of the same material, sealed in the same fashion, and then sewed and sealed to the plastic bag. The inlet and outlet tubes were prepared by sealing pieces of plastic tubing to the bag in the same manner. After the substances to be transferred were

placed in the dry box, the opening was sealed by folding the plastic and placing it between two rigid rods held tightly together by spring clamps.

Dried nitrogen was usually passed through the bag for several hours prior to use. This is standard practice even with commercial dry boxes; its purpose is to remove moisture which has been adsorbed inside the box. When the dry box is in use, a slow nitrogen flow is continuously passed through.

The efficiency of the plastic dry box is shown in Table I. A weighed amount of magnesium perchlorate was placed inside the plastic dry box and a second sample was exposed to the atmosphere immediately outside of the dry box. At the end of 6 hours, the amount of water absorbed inside the dry box was 7% of that absorbed by the sample exposed to the atmosphere. Similar results have been obtained by the authors with commercial dry boxes under the same conditions.

Table I. Water Absorption Data

Time, Hr.	Water Absorbed, G. H_2O/G. $Mg(ClO_4)_2$ Inside	Outside
2	0.0007	0.0142
4	0.0012	0.0213
6	0.0019	0.0268

It is not necessary to sew the plastic bag together, although this does give added strength to the bag. A number of plastic cements are commercially available and several of these, such as cement for Plexiglas and Lucite and Pliobond cement, have been successfully used to seal plastic dry boxes together.

The authors have used plastic dry boxes without gloves to perform simple transfers of materials. The plastic bag is sufficiently pliable to transfer a solid from one container to another.

Rubber gloves have been used in place of the plastic ones, but they are more difficult to seal to the plastic bag than the plastic gloves. The advantage of rubber gloves is that they are commercially available from several sources.

A "tinker toy" type frame can be mounted inside the plastic dry box. This arrangement keeps the bag from collapsing when the nitrogen flow is discontinued.

In the initial work on plastic dry boxes a "flour sack" type of tie was used to close the opening of the plastic dry box. A piece of rubber or glass tubing was placed in the opening and the plastic bag was then tied around it. The piece of tubing through the opening was used as the exit tube in the early work. This arrangement was satisfactory, but is not as efficient as the method shown in Figure 1.

The plastic dry box described is not intended to completely replace the metal and glass dry boxes that are commercially available today. Its extremely low cost (several dollars), however, should encourage the use of dry box techniques in procedures where they are desirable, but are not now used because of the high cost of commercial dry boxes. Other workers will probably modify the apparatus described in this article to satisfy their individual needs.

Stable Starch Solutions for Iodometry

Albert C. Holler, Twin City Testing and Engineering Laboratory, St. Paul 14, Minn.

STARCH indicator solution has wide usage in the analytical laboratory. Because of its instability in aqueous solution (mold growth, etc.) it is usually prepared fresh as needed. Many times this is inconvenient—i.e., in the field.

Preservatives recommended for aqueous starch solutions include mercuric iodide (*3*), thymol (*4*), and glycerol (*2*). This paper describes a stable starch indicator solution made by using formamide ($NCONH_2$) as the solvent for the starch.

REAGENTS

Soluble starch, Mallinkrodt Chemical Works.

Formamide, obtained through the courtesy of the Polychemicals Department, E. I. du Pont de Nemours & Co., is a clear, slightly viscous liquid with a faint ammonia odor and a boiling point of 210° C. It is completely miscible with water, methanol, ethanol, dioxane, and other solvents. It is an inexpensive reagent, commercially available in high purity.

PREPARATION AND PROPERTIES OF STARCH-FORMAMIDE SOLUTIONS

The starch solutions are prepared by first heating the formamide to 100° to 110° C. (in a hood) and stirring in a slurry of the required amount of "soluble" starch. A 5% solution is prepared by pouring a slurry of 5 grams of soluble starch and 30 ml. of cold (room temperature) formamide with stirring into 65 ml. of hot (100° to 110° C.) formamide. The starch dissolves within 1 minute after addition. The solution is ready for use as soon as it cools to room temperature.

Starch solutions as concentrated as 10% are very viscous and not readily handled by dropping bottles; 3 to 5% solutions are very suitable as an indicator solution, but solutions of different concentrations may be made up as desired.

Formamide differs from water in that it dissolves the starch, forming a clear solution of medium viscosity. On the addition of a starch-formamide solution to water, the starch is completely dissolved in the water, no doubt because of the highly polar characteristics of the formamide. When 10 ml. of a 10% starch solution was added to 50 ml. of water, there was no separation of the starch.

DISCUSSION

The sensitivity of a starch-formamide indicator solution toward iodine is the same as for fresh aqueous solutions. Two drops of a 5% starch-formamide solution imparts an intense blue color (not violet or reddish violet) to the iodide-iodine test solution (*1*).

In order to test the reactivity of formamide with iodine, a series of iodine titrations was run using the same volume of iodine solution but adding varying amounts of formamide to the iodine solution before the titrations with sodium thiosulfate (Table I). Formamide does not combine with appreciable amounts of iodine, until approximately 10 ml. of the reagent are in excess. In iodometry this would not be encountered and the error would be negligible, because at most 0.5 ml. of the starch-formamide indicator solution would be used.

The stability of the formamide-starch solution is excellent. After 8 months a 5% starch solution showed no evidence of mold growth, discoloration, or precipitation of the starch, and still retained its sensitivity toward iodine, giving an intense blue adsorption complex (not reddish violet or violet).

A 5% starch-formamide solution was inoculated with a mold (probably aspergillus) that was found growing in an aqueous starch solution. No growth of the mold was noted even after 2 months' incubation.

Table I. Reaction of Formamide with Iodine

(10.00 ml. of 0.0105*N* iodine solution taken)

Amount of Starch Solution	Sodium Thiosulfate Used, Ml.	Difference Compared with Aqueous Starch, Ml.
Fresh aqueous 1% starch solution (1 ml.)	10.30	....
5% starch-formamide solution		
Fresh, 2 drops	10.30	±0.00
6 months old, 2 drops	10.31	±0.01
1 week old, 2 drops	10.31	±0.01
5 ml. formamide added in excess	10.29	−0.01
10 ml. formamide added in excess	10.20	−0.10
30 ml. formamide added in excess	10.01	−0.29

LITERATURE CITED

(1) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," p. 618, rev. ed., Macmillan, New York, 1943.
(2) Nordling, W. D., *Chemist Analyst*, 42, 70 (1953).
(3) Tomicek, O., "Chemical Indicators," p. 225, Butterworth Scientific Publications, London, 1951.
(4) Vogel, A. I., "Quantitative Inorganic Analysis," p. 332, 2nd ed., Longmans, Green, London, 1951.