On Both Sides of the Atlantic

THE resurging interest in analytical chemistry in the United States is matched by similar developments in Great Britain.

The Chemical Trade Journal and Chemical Engineer reports a very successful display of apparatus used in chemical analysis held at the Science Museum in South Kensington. Originally the display was planned for the benefit of the two hundred analysts attending the Royal Institute of Chemistry (London Section) Summer School in Analytical Chemistry. It was later decided to present this small exhibition in a form suitable for the general public.

Considerable ingenuity was shown in the type of displays. There was, for example, a push-button demonstration of the principles of radiochemical analysis in which visitors were able to bring radioactive specimens, in turn, in front of a device for measuring their activity. The principles of microbiological analysis were shown through the use of apparatus well known to analysts.

The display was arranged through the cooperation of leading instrument manufacturers, government departments, and the senior staffs in some of London's technical colleges. According to reports, the exhibition attracted large crowds and aroused considerable interest.

The success of this project in London leads us to suggest the possibility of a similar display in New York at the time of the Diamond Jubilee Meeting of the A.C.S. and the meetings of the International Union of Pure and Applied Chemistry and the International Congress of Pure and Applied Chemistry in the fall of 1951.

Possibly the Scientific Apparatus Makers Association might take the leadership in planning such an exhibition with the support of the Division of Analytical Chemistry of the A.C.S. and other groups directly interested in publicizing the work of the analyst. This opportunity should not be lost, and if something really worth while is to be accomplished plans must be made immediately.

Chemistry and Industry, the official publication of the Society of Chemical Industry, in an editorial in the September 2 issue discussing the renaissance of analytical chemistry states:

The increased interest in analysis has been encouraged by the development of new physical methods involving the use of spectrographs, polarographs, absorptiometers, and such instruments, and by the new methods of bacteriological assay for antibiotics and vitamins and of radiochemical estimations. One of the most skilled branches of analysis is that of the microanalyst, whose handling of ingenious apparatus and techniques remains a wonder to the uninitiated. It surprised us to hear that in certain colleges all students are taught their routine inorganic qualitative analysis using a micro- or semimicrotechnique, thereby saving both reagents and time.

The public analyst has always been of necessity one of the chief upholders of the analytical branch of chemistry, but the new place that analysis is taking has led to the appearance of a number of analytical experts in industry and, where it is more surprising and more welcome, in academic laboratories. These experts are analysts first and foremost, and are analysts because they believe in the art of analysis for its own sake and not as the tedious confirmation of a successful synthesis . . .

S.A.M.A.'s Standardization Committee

WE trust that the renewed interest in standardization indicated at the recent mid-year meeting of the Scientific Apparatus Makers Association will result in more practical accomplishments than have been made in the past despite herculean efforts by John Marshall Roberts, former vice president of S.A.M.A.

Under the chairmanship of T. M. Mints, president of E. H. Sargent & Company, the standardization committee will center its attention on manufacturers rather than dealers in an effort to find areas of agreement for the elimination from manufacturers' catalogs of slow-moving items which plague both manufacturers and dealers. Efforts will also be made to cut down the number of sizes and shapes, in order to alleviate the troublesome stock and inventory problems of dealers.

The AMERICAN CHEMICAL SOCIETY is cooperating. When the complete report of the Mints committee is ready it will be forwarded to W. D. Collins, who will review the S.A.M.A. recommendations with the Standardization Committee of the Society. Users, dealers, and manufacturers—all should benefit from a realistic evaluation of what can be eliminated from the catalogs of manufacturers and dealers.

Number 3 Is Analytical

NUMBER 3 in the *Advances in Chemistry Series* has now made its appearance. "Analytical Methods in the Food Industry" is a collection of the papers presented at the Symposium on Analytical Methods in the Food Industry held by the Divisions of Analytical Chemistry and Agricultural and Food Chemistry of the AMERICAN CHEMICAL SOCIETY at the 115th national meeting in San Francisco, March 28 to April 1, 1949.

The latest in the series is priced at $1.50 per copy, and your order with check or money order should be sent to the AMERICAN CHEMICAL SOCIETY, 1155 Sixteenth St., N. W., Washington 6, D. C., attention Special Publications Department.

The reception given the *Advances in Chemistry Series* is most gratifying. These monographs are recognized universally as a new and important addition to the permanent literature. A limited number of copies of "Agricultural Control Chemicals" (No. 1, $2.50) and "Chemical Factors in Hypertension" (No. 2, $1.00) are still available.

TETRAETHYLLEAD IN GASOLINE

Rapid Determination of Tetraethyllead in Aviation Gasoline

V. A. SMITH, W. E. DELANEY[1], W. J. TANCIG, AND J. C. BAILIE

Standard Oil Company (Indiana), Whiting, Ind.

A new and rapid method for the determination of tetraethyllead in aviation gasoline is based on reaction of alcoholic silver nitrate with tetraethyllead to form metallic silver. The silver, present as a colloidal suspension, is determined by turbidimetric methods using a photoelectric colorimeter. The method is applicable to determination of the tetraethyllead content of aviation fuels and motor-fuel stocks sweetened with copper chloride but cannot be used with doctor-sweetened stocks because of interference by free sulfur or polysulfides.

THE determination of tetraethyllead (TEL) in gasoline has been the subject of numerous investigations. Chemical methods which have been developed for this determination (8) all require several hours elapsed time. Satisfactory methods involving polarographic (3, 6) or x-ray absorption (11) techniques have been described, but equipment for these methods is not generally available in control laboratories. A rapid method utilizing readily available equipment would therefore be desirable.

The Russian worker, Bykhovskaya (4), observed that tetraethyllead reacts with silver nitrate in alcohol solution to produce a dark-colored solution. The reaction was utilized to estimate small amounts of tetraethyllead in air by passing the sample of air through a tube of silica gel moistened with alcoholic silver nitrate and measuring the length of the darkened gel. This reaction appeared to provide the basis for a rapid method for the determination of tetraethyllead in gasolines. Preliminary experiments confirmed the reaction and provided the stimulus for further investigation; this resulted in the rapid turbidimetric method herein described.

The reaction of silver nitrate with tetraethyllead involves the formation of ethyl silver (7):

$$(C_2H_5)_4Pb + AgNO_3 \longrightarrow (C_2H_5)_3PbNO_3 + C_2H_5Ag \quad (1)$$

The latter is thermally unstable and decomposes to form metallic silver and free radicals.

$$C_2H_5Ag \longrightarrow C_2H_5\cdot + Ag$$
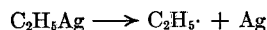
The silver liberated in alcoholic solution gives a yellow- to brownish-black coloration, the optical density of which can be determined by means of a photoelectric colorimeter. By comparison with a standard calibration curve, the tetraethyllead content of the fuel can be determined.

EXPERIMENTAL

An Evelyn photoelectric colorimeter with a 520-mμ filter was used in the developmental work on this method. Reagents employed were c.p. 95% ethyl alcohol and a saturated (approximately 5%) solution of c.p. silver nitrate in this alcohol. In

[1] Present address, Griffin Laboratories, Chicago, Ill.

making up synthetic samples used in preparing the calibration curve, iso-octane (2,2,4-trimethylpentane) was employed.

Procedure. On the basis of the estimated tetraethyllead content, portions of the gasoline sample to be tested are accurately diluted with 95% ethyl alcohol to a tetraethyllead concentration between 0.10 and 0.50 ml. per gallon. With a pipet 20.0 ml. of the diluted sample are then introduced into a 250-ml. glass-stoppered Erlenmeyer flask. From a graduated cylinder 100 ml. of silver nitrate solution are added to the flask; a timer is started immediately after the addition of the silver nitrate. The flask is stoppered and shaken for a few seconds. The solution is then transferred to a colorimeter cell, the colorimeter having first been adjusted to zero with the silver nitrate solution. Optical density is read at the end of the first, second, third, and fifth minutes indicated by the timer. The reaction is rapid and, in the absence of interfering substances, the drift of the colorimeter reading is usually slight. The reading for comparison with the calibration curve is best taken at the end of the second minute. The optical density observed is compared with a standard calibration curve, and the amount of tetraethyllead present is determined as milliliters per gallon of original gasoline. A complete determination can normally be made within 10 minutes.

Preparation of Calibration Curve. For use with the Evelyn colorimeter, a calibration curve covering the range from 0.10 to 0.50 ml. of tetraethyllead per gallon has been found satisfactory and convenient. Since the tetraethyllead content of most gasolines lies between 1 and 5 ml. per gallon, this calibration range generally permits dilution of unknown samples by the pipetting of 10-ml. portions into 100-ml. volumetric flasks.

Iso-octane containing 3.0 ml. of tetraethyllead per gallon was used as the basic standard. Into 100-ml. volumetric flasks there were transferred aliquots of 3, 5, 7, 10, 13, and 17 ml. of this standard, and these volumes were diluted to the mark with 95% ethyl alcohol. The resulting solutions contained 0.09, 0.15, 0.21, 0.30, 0.39, and 0.51 ml. of tetraethyllead per gallon, respectively. Portions (20 ml.) were treated with silver nitrate solution as described in the procedure, and the optical densities were successively measured in the colorimeter and plotted against the known tetraethyllead concentration. A typical calibration curve is shown in Figure 1.

RESULTS AND DISCUSSION

Results of analyses of samples of iso-octane containing known amounts of tetraethyllead are shown in Table I. These samples were prepared and submitted to the analyst as unknowns. The extreme deviation was 0.10 ml. per gallon on one determination, and

the average deviation for the eight samples was ±0.026 ml. per gallon. Inasmuch as the results obtained were sufficiently accurate, the method was considered adequate for control work. There was no dye interference in the dyed gasolines at the dilutions used.

Results obtained with several aviation and motor fuels are shown in Table II. For comparison, determinations by the standard A.S.T.M. method D 526 (1) are also shown. The A.S.-T.M. samples were distributed by Committee D-2, Subcommittee A, Section VII, for cooperative testing of certain variables in the gravimetric procedure D 526. Table II shows that the aviation gasolines gave results which were in close agreement with those obtained by the A.S.T.M. method. However, motor fuels analyzed by the silver nitrate method consistently gave high re-
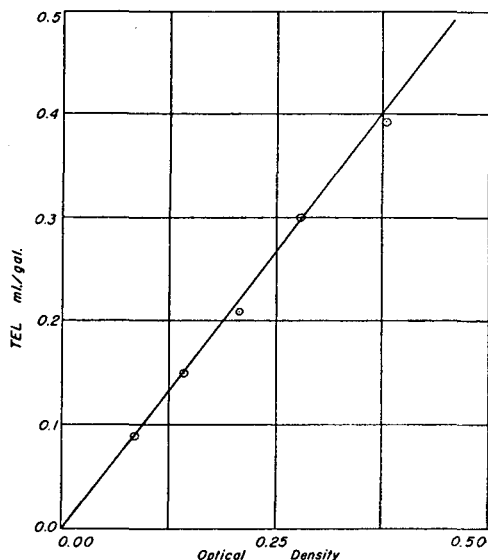

Figure 1. Typical Calibration Curve

sults. This was due to the presence of an interfering substance which reacted slowly with silver nitrate and gave an unstable and high colorimeter reading.

In every case these motor fuels were known to contain components which had been subjected to the "doctor" treating process. This process normally involves treating the stock with sulfur and alkaline sodium plumbite solution which converts mercaptans to alkyl disulfides; in the presence of excess sulfur, higher polysulfides may be formed. In stocks which are sweetened by the copper chloride process, the mercaptans are converted to disulfides but no polysulfides are formed. Copper chloride-sweetened stocks containing known quantities of tetraethyllead gave the expected value by the silver nitrate procedure. This pointed to polysulfides or free sulfur in doctor-sweetened stocks as the interfering substances responsible for the high values. In confirmation, it was found experimentally that small quantities of free sulfur or polysulfides, dissolved in iso-octane containing tetraethyllead, caused the same characteristic colorimeter drift and high tetraethyllead values as had previously been obtained with motor fuels containing doctor-sweetened components.

Polysulfides are known to be rather unstable compounds in which sulfur atoms are loosely held in combination. It is assumed, therefore, that silver liberated in the colloidal state in the reaction between silver nitrate and tetraethyllead would react with the loosely held sulfur atoms to form silver sulfide. Such a reaction would continue until the reactive sulfur was removed and a stable polysulfide (probably a disulfide) was formed. The course of this reaction would account for the gradual drift of the colorimeter reading and the high tetraethyllead result.

Many attempts have been made to remove the interfering substances from fuels containing doctor-sweetened stock without affecting the tetraethyllead, but all have been unsuccessful. Oxidizing and reducing agents under various conditions either partially destroyed the tetraethyllead or had no effect on the interfering substances. Treatment with various reagents including triethylenetetramine, chloramine-T, metallic mercury, lime and hydrogen sulfide (5), sodium hydroxide and hydrogen peroxide (10), alkali-metal stannite solution (2), and piperidine (9) all failed to accomplish the desired end. Adsorption on activated charcoal, Attapulgus clay, or aluminum oxide was likewise unsuccessful.

CONCLUSION

The turbidimetric technique provides a simple and rapid procedure for the determination of tetraethyllead in aviation gasolines or motor fuels which do not contain free sulfur or polysulfides. With established calibration curves, an average analysis can generally be made in less than 10 minutes. Results on aviation fuels check closely with A.S.T.M. method D 526.

Table I. Analysis of Iso-octane Samples of Known Tetraethyllead Content

TEL Concn., Ml./Gal.

Known	Found	Deviation, %
0.20	0.20	0.00
0.40	0.38	−0.02
1.04	1.03	−0.01
1.20	1.24	+0.04
	1.18	−0.02
	1.27	+0.07
1.80	1.73	−0.07
	1.80	0.00
	1.80	0.00
2.00	2.00	0.00
	2.00	0.00
	1.90	−0.10
2.40	2.43	+0.03
3.04	3.05	+0.01

Av. ±0.026

Table II. Results of Tetraethyllead Analysis by Silver Nitrate Method and A.S.T.M. Method

TEL Concn., Ml./Gal.

Sample	A.S.T.M. method	AgNO₃ method	Deviation, %
Aviation fuel No. 1	2.99	3.02	+0.03
2	2.92	2.92	0.00
3	3.75	3.79	+0.04
4	3.68	3.66	−0.02
5	3.74	3.75	+0.01
6	3.61	3.60	−0.01
Motor fuel, Type A, No. 1	1.30	2.17	+0.87
A No. 2	2.09	2.35	+0.26
B No. 1	1.89	2.06	+0.17
B No. 2	2.91	3.25	+0.34
A.S.T.M. sample PPC-1	4.36	4.34	−0.02
PPC-2	2.99	3.45	+0.46
PPC-3	0.99	0.95	−0.04
PPC-4	1.97	2.10	+0.13

LITERATURE CITED

(1) Am. Soc. Testing Materials, "A.S.T.M. Standards on Petroleum Products and Lubricants," Committee D-2, 1948.
(2) Ayers, G. W., and Lyon, P., U. S. Patent 2,435,732 (Feb. 10, 1948).
(3) Borup, R., and Levin, H., Proc. Am. Soc. Testing Materials, 47, 1010 (1947).
(4) Bykhovskaya, M. S., Gigiena i. Sanitariya, 10, 17 (1945).
(5) Espach, R. R., Blade, O. C., and Rue, H. P., Refiner Natural Gasoline Mfr., 13, 65 (1934).
(6) Frediani, H. A., and Bass, L. A., Oil Gas J., 39, No. 20, 51 (1940).
(7) Krause, E., and von Grosse, A., "Die Chemie der metallorganischen Verbindungen," p. 792, Berlin, Gebruder Borntraeger, 1937.
(8) Lykken, L., Treseder, R. S., Tuemmler, F. D., and Zahn, V., IND. ENG. CHEM., ANAL. ED., 17, 353 (1945).
(9) Riding, R. W., and Thomas, J. S., J. Chem. Soc., 1923, 3271.
(10) Szeberenyi, P., Z. anal. Chem., 78, 36 (1949).
(11) Vollmar, R. C., Petterson, E. E., and Petruzzelli, P. A., ANAL. CHEM., 21, 1491 (1949).

Rapid Polarographic Determination of Tetraethyllead in Gasoline

KENT A. HANSEN, THOMAS D. PARKS[1], AND LOUIS LYKKEN
Shell Development Company, Emeryville, Calif.

A rapid, direct polarographic method for determination of tetraethyllead in gasoline is described. The sample is dissolved and the tetraethyllead is decomposed in anhydrous Cellosolve containing hydrogen chloride, and the lead ions in the resulting solution are determined directly by the polarograph. The method is applicable to tetraethyllead in gasolines in the range of 0.5 to 8 ml. per gallon, and is generally accurate to within ±3% of the lead content in freshly prepared blends. Aged gasolines containing high concentrations of unsaturates and peroxides may give appreciable errors. A single determination requires about 30 minutes, but a series of 5 determinations requires only 1 hour.

IN RECENT years, there has been a sustained interest in finding rapid and accurate methods for the determination of tetraethyllead in gasoline. Lykken, Treseder, Tuemmler, and Zahn (5) recently reviewed the existing chemical methods and noted that a common disadvantage of all the methods was the amount of time required per analysis; they recommended two new direct evaporation procedures which gave quantitative analysis of lead in all types of gasolines but which required 3 or 4 hours per sample, depending on the type of base stock.

Perhaps the most rapid method for the determination of tetraethyllead in gasoline is the x-ray absorption method of Sullivan and Friedman (8), which uses a Geiger counter to measure the x-ray absorption of a leaded gasoline sample and which gives precise and accurate results, even by unskilled workers, in an elapsed time of 5 minutes per sample. One disadvantage of the x-ray for plant control is the expensive equipment required. The Widmaier iodometric method (6, 9) is simple and rapid, requiring only 10 to 15 minutes per sample, but it is not sufficiently accurate for many applications. The authors' experience indicates that the Widmaier method gives slightly high results for saturated stocks and up to 10% too high values for unsaturated stocks, even when the pretreatment with 70% sulfuric acid is used as recommended by Widmaier (9) and Newman, Philip, and Jensen (6). Frediani and Bass (3) described a polarographic method for analyzing the lead solution obtained by extracting the gasoline samples with hydrochloric acid (1). Although their method permits a decrease in time per analysis in comparison with chemical methods, they pointed out that a direct determination without previous acid extraction would result in saving much additional time in gasoline analysis. The recently published method of Borup and Levin (2) is essentially a simplification and refinement of this extraction method.

This paper describes a rapid method for the direct polarographic determination of lead in a nonaqueous solution of gasoline, eliminating the extraction step. The leaded gasoline is added to a solution of hydrochloric acid in anhydrous Cellosolve (ethylene glycol monoethyl ether) with which it is miscible (7). The tetraethyllead is decomposed by heating the mixture and without further treatment the resulting solution is analyzed polarographically for lead.

APPARATUS

Polarograph. A Sargent Model XX polarograph and a unitized dropping mercury electrode and cell assembly (4) pro-

---

vided with temperature control and operated at 25° ± 0.5° C. were used in all analyses.

REAGENTS

Cellosolve–Hydrogen Chloride Electrolyte. Prepare by passing anhydrous hydrogen chloride into Cellosolve until the solution is approximately 1 N, as determined by diluting a portion with water and titrating with standard base.

Tetraethyllead Standards. Add sufficient tetraethyllead fluid to lead-free gasoline to make solutions containing 0.5, 1.0, 4.0, and 8.0 ml. of tetraethyllead per gallon. Determine the exact concentration by A.S.T.M. D 526 (1) or method of equal accuracy (5).

Nitrogen. Tank nitrogen containing less than 0.5% oxygen.

PROCEDURE

By means of a pipet or buret, measure exactly 10.0 ml. of Cellosolve–hydrogen chloride electrolyte and 3.0 ml. of leaded gasoline into a 25-ml. borosilicate glass volumetric flask. Heat the flask on a steam bath for 15 to 20 minutes, cool to room temperature, and transfer a portion of the solution to the polarographic cell. Adjust the temperature of the cell to 25° C. Purge the cell by passing nitrogen through the liquid in the cell for 5 minutes at a rate of 150 ml. per minute. Adjust the mercury pressure so that a drop time of 4 seconds per drop is obtained with 0.4 volt applied to the electrodes. Make a polarogram covering the range of 0.2 to 0.5 volt. Measure the height of the polarographic wave and from a calibration curve determine the concentration of tetraethyllead in the sample.

EXPERIMENTAL

In order to develop a method that would be applicable to the analysis of any gasoline, it was considered necessary to base the determination on the reduction of the lead from the tetraethyllead compound and not from any other reducible substance in the Ethyl fluid. Thus an electrolyte was desired which would not only dissolve and decompose the tetraethyllead but also would permit the direct polarographic determination of the resulting lead ion in the same medium without further treatment. It was found that a 1 N solution of hydrochloric acid in Cellosolve was miscible with about 20% of its volume of leaded gasoline, and that it gave a diffusion current for lead ion after heating a short time to decompose the tetraethyllead. Later, it was found that a better reagent with greater solubility for gasoline was obtained by passing anhydrous hydrogen chloride gas into Cellosolve until it was about 1 N as determined by titration. This electrolyte was miscible in all proportions with gasoline. No apparent decomposition took place when a leaded gasoline was mixed with the Cellosolve–hydrogen chloride solution at room temperature.

However, experiments showed that heating at steam temperature for 15 minutes was sufficient to decompose the tetraethyllead completely and to make the lead available for polarographic analysis. Consistent, reproducible results were obtained when the heating time was maintained between 15 and 20 minutes, but low wave heights were found with longer heating, due possibly to precipitation of lead chloride. Using a heating time of 15 to 20 minutes, the elapsed time for one sample was about 30 minutes, but a group of five samples was completed in 1 hour. The heating was carried out in 25-ml. volumetric flask to give a degree of refluxing, thus minimizing volume changes due to evaporation and making further dilution to volume unnecessary.

A number of leaded gasoline samples having a variety of hydrocarbon types were analyzed by this method and at the same time by A.S.T.M. D 526 (1). The results, summarized in Table I, agreed in most cases within 3%, with a precision of better than 1%. Figure 1 depicts a typical polarogram and shows the method used to measure the wave height. Calculations were minimized by preparing the calibration curve directly in milliliters of tetraethyllead per gallon. A straight-line calibration was obtained. While the most accurate concentration range is from 0.5 to 8.0 ml. per gallon, samples containing as little as 0.1 ml. per gallon were estimated with an accuracy of 5%.

DISCUSSION

Several solvents were tested in an effort to find a medium in which leaded gasoline could be examined polarographically. Gasoline was found to be insufficiently soluble for this purpose in the following solvents mixed with the required amount of concentrated hydrochloric acid: ethyl and isopropyl alcohols, methyl or ethyl Carbitol (diethylene glycol monomethyl or monoethyl ether), benzene–isopropyl alcohol mixture, and methyl Cellosolve (ethylene glycol monomethyl ether). Gasoline was found to be very soluble in dioxane and in ethyl Cellosolve, but the latter was preferred for polarographic work because of the rapid formation of peroxide in dioxane (7). Samples of a leaded gasoline were dissolved in 0.1 N solution of tetra-n-butyl ammonium iodide in Cellosolve and, without any further treatment, were examined polarographically over the voltage range of 0.0 to −2.0 volts. Waves were obtained at an applied potential of −1.30 and −1.70 volts, but these were found to be due to ethylene bromide and ethylene chloride, respectively, and not to lead. Because ethylene dibromide is a component of both motor and aviation tetraethyllead fluids, it could possibly be used in an indirect indication of the tetraethyllead present in certain gasoline
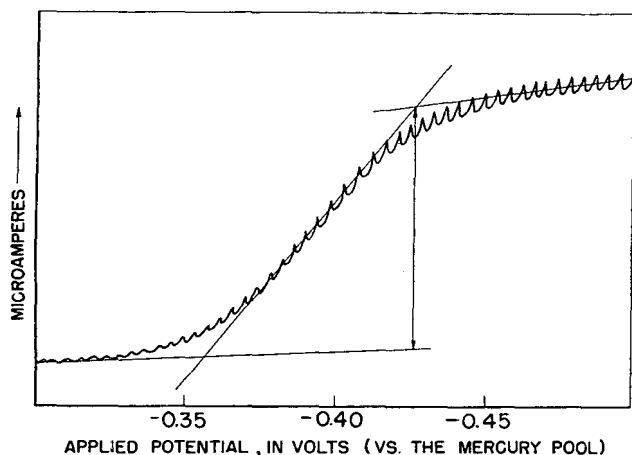
samples. Such a procedure would be extremely rapid and would be particularly useful in control or blending work where samples of the added batch of fluid are available for making a calibration curve. However, it would have very limited application in the analysis of unknown samples.

Table I. Tetraethyllead Results for Various Types of Gasolines by Rapid Polarographic Method

| Gasoline Sample | TEL Content, Ml. per Gal. | | |
	A.S.T.M. D 526	Polarographic	Difference
Motor	0.54	0.54	
	0.56	0.58	
Av.	0.55	0.56	0.01
Motor, low sulfur	1.51	1.52	
	1.51	1.52	
Av.	1.51	1.52	0.01
Motor, low sulfur	0.86		
	0.89	0.84	
	0.84	0.86	
Av.	0.86	0.85	0.01
Motor, high sulfur	1.68	1.69	0.01
Aviation I	4.51	4.61	
	4.49	4.61	
Av.	4.50	4.61	0.11
Aviation II	3.83		
	3.89	4.02	
	3.88	4.13	
Av.	3.86	4.07	0.21
Aviation III	3.82	3.92	
	3.84	3.92	
Av.	3.83	3.92	0.09
Aviation IV	4.01		
	4.01	4.02	
	4.04	4.00	
Av.	4.02	4.01	0.01
Special aviation	5.07		
	5.03	5.0	
Av.	5.05	(5.0)	0.05
Special aviation	8.67	8.40	
	8.65	8.40	
Av.	8.66	8.40	0.26
Aviation	3.44	3.57	
	3.45	3.57	
Av.	3.45	3.57	0.12
Thermally cracked	2.12	2.17	
	2.13	2.17	
Av.	2.13	2.17	0.04
Catalytically cracked	2.28	2.12	
	2.27	2.10	
Av.	2.28	2.11	0.17

In the analysis of aged gasoline stocks containing high concentrations of unsaturated hydrocarbons and/or high concentrations of organic peroxides errors as large as 10% have been obtained. No satisfactory explanation for this error was found. A portion of the error, however, can be attributed to the poorer polarographic reduction waves obtained when the unsaturated hydrocarbons or peroxides are present. Some knowledge of the type of sample being analyzed should be available before the results of the polarographic method can be properly evaluated. For freshly prepared gasoline stocks, these difficulties are generally avoided and an accuracy within ±3% can be obtained.

LITERATURE CITED

(1) Am. Soc. Testing Materials, "Standards on Petroleum Products and Lubricants," A.S.T.M. Designation D 526–42, October 1947.
(2) Borup, R., and Levin, H., Am. Soc. Testing Materials Proc., 47, 1010 (1947).
(3) Frediani, H. A., and Bass, L. A., Oil Gas J., 38, No. 20, 51 (1940).
(4) Lykken, L., Pompeo, D. J., and Weaver, J. R., IND. ENG. CHEM., ANAL. ED., 17, 724–8 (1945).
(5) Lykken, L., Treseder, R., Tuemmler, F. D., and Zahn, V., Ibid., 17, 353–60 (1945).
(6) Newman, L., Philip, J. F., and Jensen, A. R., ANAL. CHEM., 19, 451–3 (1947).
(7) Parks, T. D., and Hansen, K. A., Ibid., 22, 1268 (1950).
(8) Sullivan, M. V., and Friedman, H., IND. ENG. CHEM., ANAL. ED., 18, 304–6 (1946).
(9) Widmaier, O., Luftfahrt-Forsch., 20, 181 (1943).

Figure 1. Typical Polarogram of Lead Ion in Cellosolve–Hydrogen Chloride Electrolyte

Sensitivity, 0.036 μa. per ml.
Temperature, 25° C.
Tetraethyllead concentration, 2.1 ml. per gallon

A Direct-Reading Polarograph

For Determination of Tetraethyllead in Gasoline

E. B. OFFUTT AND L. V. SORG

Research Department, Standard Oil Company (Indiana), Sugar Creek, Mo.

The successful investigation of antimony as a pilot ion in the polarographic determination of lead has made possible the construction of an instrument by which the lead concentration of a gasoline may be read directly from a calibrated dial while the dropping mercury electrode is polarized in the hydrochloric acid extract (D 526–48T) from the oil. Predetermined potentials are applied through a sequence of push-button operations. Compensation of the resulting currents is accomplished by adjustment of potentiometers using a galvanometer as a null instrument; the last such adjustment gives the reading of tetraethyllead concentration in milliliters per gallon. Provision has been made for rapid standardization of the instrument and repetition of the push-button sequence for check determinations. Comparison of this polarographic method with the standard A.S.T.M. method for the determination of tetraethyllead in gasoline shows that the polarographic method is at least equivalent to the A.S.T.M. method with respect to both accuracy and reproducibility.

A NUMBER of analytical procedures have been proposed for the polarographic determination of tetraethyllead (TEL) in gasoline (*2, 4*). These procedures involve tetraethyllead decomposition by hydrochloric acid, subsequent extraction of the resulting lead chloride in a manner similar to that described by Calingaert and Gambrill (*3*), and analysis of the extract by means of current measurements in a polarographic cell containing a dropping mercury electrode. Inasmuch as lead is the only metallic element present in the extract solution from the gasoline, the solution readily lends itself to polarographic analysis; the lead concentration in such extracts is generally in the range particularly suitable for this type of analysis.

The method of Calingaert and Gambrill is currently the tentative standard method of the American Society for Testing Materials (*1*). This method, commonly referred to as the A.S.T.M. method, consists of acid extraction of lead from the gasoline, gravimetric determination of the lead as lead chromate, and conversion to milliliters of tetraethyllead per gallon (3785 ml.) of gasoline. Carbonaceous material contained in the lead extract must be destroyed by wet oxidation prior to precipitation of the lead chromate. This is a time-consuming operation from the standpoint of routine analytical procedure because of the required multiple evaporation.

Application of certain polarographic techniques for determination of the lead in the extract makes it unnecessary either to remove the carbonaceous material or to evaporate the solution. In general, therefore, a polarographic procedure offers the advantage of requiring less time for a tetraethyllead determination than the A.S.T.M. gravimetric method. However, in many of the advocated procedures for polarographic analysis, a polarogram of the solution is prepared by either manual or automatic plotting of the relationships between voltage and diffusion current that result from the effect of the lead ion, and sometimes of other ions, upon a dropping mercury electrode placed in the solution. The subsequent measurement of the lead-ion diffusion current is converted to an equivalent tetraethyllead value by reference to a previously prepared standard calibration curve or chart. Such a polarographic technique requires relatively complicated and expensive equipment.

In view of the specific nature of the lead solution obtained from the tetraethyllead extraction, it has been recognized that polarograms are not required and that a relatively simple polarograph can be employed for the analysis. In order to render the polarographic technique more suitable for routine use and to reduce it to

the simplest practical form, a new polarographic instrument has been developed. This unique instrument has been designed to indicate directly—without calculations or reference to graphs or charts—the lead content of the acid extract from a gasoline. The analytical result, obtained after performing several simple operations and adjustments, is read directly from a double-scale dial
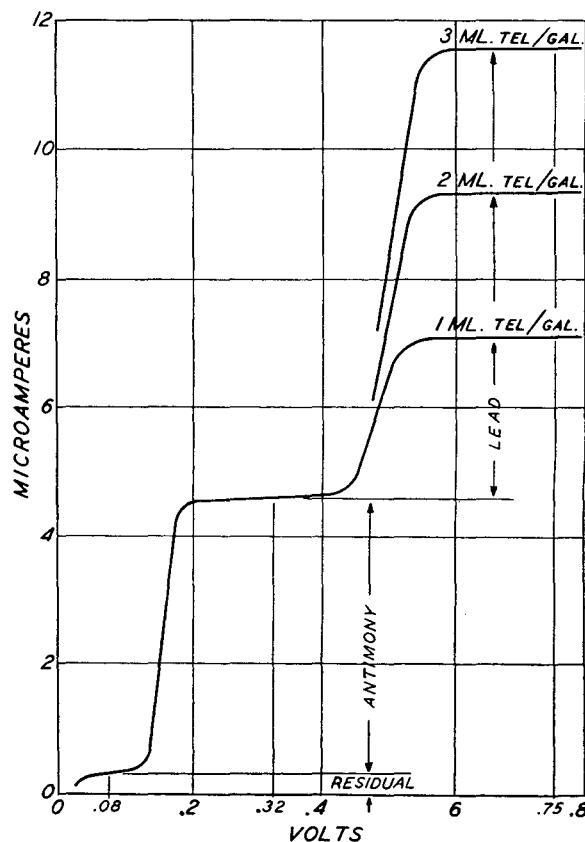

Figure 1. Relationship between Voltage Applied to Dropping Mercury Electrode and Resulting Diffusion Currents Due to Antimony and to Three Different Concentrations of Lead

calibrated for the two ranges 0 to 4 and 4 to 8 ml. of tetraethyl-lead per gallon of gasoline.

PRINCIPLE

Operation of the new polarograph is based upon the use of antimony as a pilot ion in the polarographic measurement of lead in the acid extract from gasoline. Maintaining the half-wave potential of the pilot ion below that of lead—as is the case with antimony—permits simplification of the design and use of the polarograph. Figure 1 presents a set of characteristic curves showing the relationship between voltage applied to the dropping mercury electrode and the resulting diffusion currents due to antimony and to three different concentrations of lead, equivalent to 1, 2, and 3 ml., respectively, of tetraethyllead per gallon of gasoline. The voltage values are those measured between the dropping mercury electrode and the quiet mercury pool with a hydrochloric acid supporting electrolyte. The current increase obtained by raising the voltage to 0.32 volt is the diffusion current due to antimony ions, and the next current increase obtained by raising the voltage to 0.75 is due to lead ions.

With this instrument, diffusion currents are not actually measured, but rather the magnitude of the current due to lead ions is compared with the current due to added antimony ions. As the amount of antimony added to the solution is constant, the resulting ions serve as pilot ions to which the lead may be referred. By comparing the diffusion current due to the lead, which varies in concentration, with that due to antimony, which is constant in concentration, variations due to moderate differences in mercury-dropping rate, cell temperature, acidity of the solution, and solution dilution are nullified.

The influence on lead determinations of several variables has been investigated. The mercury dropping rate may vary from 3 to 5 seconds per drop. Temperature change within the range of 20° to 30° C. has no measurable effect upon the accuracy of the analyses. No measurable effect is evident when the acidity of the solution to be analyzed is increased or decreased by 40% from the typical value of 1.2 N. In the final preparation of the solution to be analyzed, exact dilution is not required.

DESCRIPTION

The new direct-reading polarograph is illustrated in Figure 2. [In accordance with the long established policy of Standard Oil Company (Indiana) of making its new developments generally available, it is expected that arrangements will be consummated in the near future for the licensing of an established instrument company to manufacture and sell this type of polarograph.]

The instrumentation is housed in a case measuring approximately 10 inches wide, 14 inches deep, and 8 inches high. Two push buttons located just above the extreme left knob are used to operate sensitivity switches in the galvanometer circuit, the circuit being open when these buttons are released. The knob for adjusting the galvanometer "zero" to the center of the galvanometer scale is located on the top rear of the galvanometer case. The group of four push buttons are switches to alter the electrical circuits during a determination. The first knob from the left on the front of the panel is a multipurpose switch used for turning on the working battery and the pilot lights, cutting the galvanometer into the circuit, and selecting the range for lead concentrations. The remaining knobs adjust the various balancing voltages to produce a null point on the galvanometer, the final adjustment being made by the large knob connected to the calibrated dial visible directly above it. Two No. 6 dry-cell batteries are contained inside the case and may be removed through a hinged door on the instrument back. The direct-reading polarograph is a completely self-contained instrument, requiring a 115-volt alternating current source only for supplying power for pilot lamps and the galvanometer lamp.

The external part of the assembly (dropping mercury electrode and measuring cell) is of the conventional glass type, modified to incorporate certain advantages including some described by Mueller (6) and Kahan (5). The upper portion is a constant-head mercury reservoir attached by means of a short rubber sleeve to the mercury-dropping capillary. The capillary extends into the lower measuring-cell chamber, which contains the quiet mercury pool and the solution to be analyzed. The measuring cell is designed to facilitate measurement of successive samples without disassembly. The mercury-dropping rate is controlled by the bore of the capillary and the mercury head. Provision is included for purging the test solution and the measuring cell with oxygen-free nitrogen.
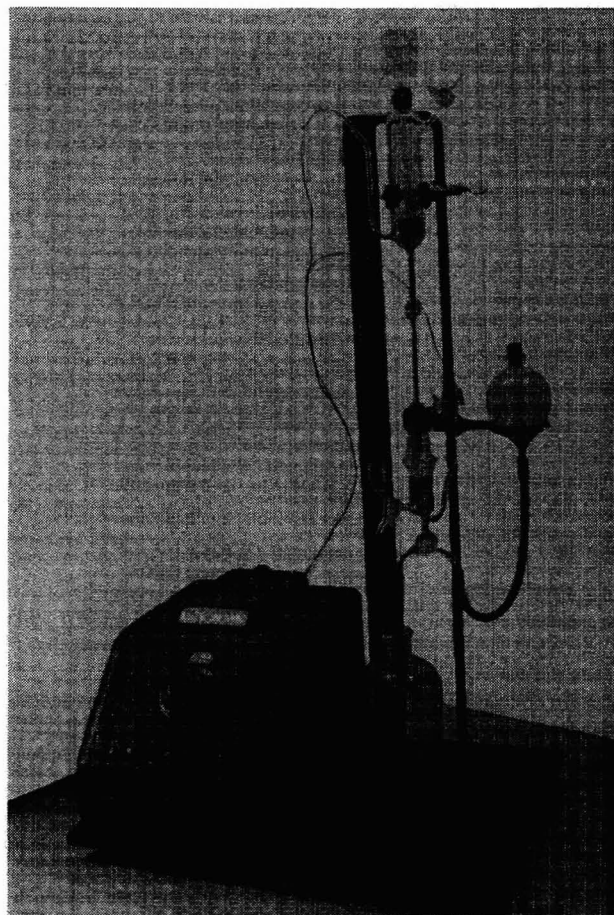

Figure 2. Direct-Reading Polarograph

Electrical System. The electrical principle of the new polarograph is illustrated in Figure 3.

The $S5$ group of switches is assembled in a gang operated by the left-hand knob (Figure 2). The positions of $S5$ govern the following functions: 1, off; 2, standardize; 3, measure tetraethyllead, 0- to 4-ml. range; 4, measure tetraethyllead, 4- to 8-ml. range. Switches $S1$, $S2$, $S3$, and $S4$ are mounted in a push-button assembly and are individually operated by a latching, inter-releasing push-button mechanism. Depressing one of the buttons latches the button down and automatically releases any other button that may have been previously latched down.

The galvanometer, G, is connected to appropriate points in the circuit by the switch action of $S5$ and the various push-button switches to serve as a null balance indicator. Resistors R_{11}, R_{12}, R_{13}, R_{14}, and R_{15} govern the effective galvanometer sensitivity in the different functions.

The transformer, T, supplies current for the galvanometer scale lamp, L_1, and dial lamps, L_2, illuminating the 0 to 4 ml. of tetraethyllead per gallon range, and L_3, illuminating the 4- to 8-ml. range.

The circuit contains three voltage divider networks: one consisting of R_2, R_{2A}, R_3, R_4, R_{4A}, R_5, and R_6; another of R_7, R_8, and R_9; and the third of R_{16}, R_{17}, R_{18}, and R_{19}. Voltages across the networks are standardized by balancing the voltage of a Weston standard cell, $S.C.$, with the voltage drop across a portion of the R_2–R_6 network; this is accomplished by adjusting the current from battery B with resistor R_1.

Taps are taken on resistors in the R_{16}–R_{19} network at 0.08, 0.32, and 0.75 volt. By means of $S2_2$, $S3_2$, or $S4_2$, these potentials may be applied across the electrodes of the measuring cell, C.

As a potential is applied to the measuring cell containing a properly prepared solution, an electrical current flows through the cell and causes a proportional voltage drop across resistor R_{10} in series with the cell. This voltage drop, which fluctuates in a regular manner because of the mercury-drop formation at the tip of the capillary, is measured by the R_7-R_8-R_9 network. The current through this network is adjusted by the positions of the sliders on R_{2A} and R_{4A} in the following manner: When S_2 is depressed to apply 0.08 volt to C, the average voltage across R_{10} (due to the measuring-cell residual current) is balanced by adjusting the R_{2A} slider so that the galvanometer swings equally to each side of zero; when S_3 is depressed to apply 0.32 volt to C, the average voltage across R_{10} (due now to the residual current plus the antimony-ion diffusion current) is balanced with the voltage drop across R_7 by adjusting R_{4A}. Thus the current through the R_7-R_8-R_9 network becomes a function of the antimony-ion diffusion current.

Depressing $S4$ applies 0.75 volt to C. The average voltage drop across R_{10} is now due to the residual current plus the diffusion current due to both antimony and lead ions. This voltage is balanced by adjusting the slider of R_8, the calibrated potentiometer to which the instrument dial is attached. The position of the slider is indicated by the dial, graduated in milliliters of tetraethyllead per gallon of gasoline, visible through the window just above the large knob attached to R_8.

The relationship between resistances R_7, R_8, and R_9 is adjusted as a part of the original instrument calibration. Following the final adjustment in a tetraethyllead measurement, the following mathematical ratios are equal:

For the 0- to 4-ml. range,

$$\frac{\text{Selected portion of } R_8}{R_7} = \frac{\text{lead-ion diffusion current}}{\text{antimony-ion diffusion current}}$$

For the 4- to 8-ml. range,

$$\frac{R_9 + \text{selected portion of } R_8}{R_7} = \frac{\text{lead-ion diffusion current}}{\text{antimony-ion diffusion current}}$$

ANALYTICAL METHOD

The extraction apparatus is the same as that described in the A.S.T.M. method (*1*).

A special pipet, which delivers the equivalent of 50 ml. of gasoline at 60° F. for various actual temperatures differing from 60° F., is used to measure the sample. The stem of this pipet carries a special scale graduated from 15.6° to 35° C. Other equipment consists of standard analytical glassware.

The standard "pilot ion" solution consists of 0.0308 mole of antimony trichloride and 200 ml. of concentrated hydrochloric acid in 1000 ml. of solution. The standard lead solution consists of 1.875 grams of C.P. lead chloride dissolved in distilled water and diluted to 1000 ml.; 10 ml. are equivalent to the lead contained in 50 ml. of gasoline having 1 ml. tetraethyllead per gallon. The maxima-suppressor solution consists of 1 gram of methylene blue dissolved in 1000 ml. of distilled water. The hydrochloric acid used is C.P. concentrated of 1.18 to 1.19 specific gravity. The mercury for the capillary electrode is C.P. triple-distilled, dried, and stored in glass.

Table I. Accuracy and Repeatability of Polarographic Method

(Ml. of TEL per gallon of iso-octane)

| Known | Found | | | | |
	1st	2nd	3rd	4th	Av.
0.50	0.50	0.49	0.48	0.52	0.50
0.90	0.91	0.89	0.89	0.90	0.90
1.20	1.20	1.21	1.19	1.20	1.20
1.90	1.88	1.88	1.88	1.89	1.88
2.50	2.51	2.50	2.50	2.48	2.50
2.90	2.91	2.92	2.91	2.92	2.92
3.40	3.41	3.40	3.39	3.40	3.40
3.90	3.89	3.92	3.91	3.93	3.91
5.95	5.96	5.96	5.95	5.93	5.95
7.90	7.92	7.94	7.92	7.91	7.92

Procedure. The tetraethyllead in a sample of gasoline equivalent to 50 ml. at 60° F. is decomposed with hydrochloric acid and is extracted in accordance with the procedure of the A.S.T.M. (*1*). Into the combined acid and aqueous extracts, contained in a 250-ml. graduated glass-stoppered cylinder, are pipetted 5.00 ml. of the standard antimony pilot-ion solution and

5 ml. of maxima-suppressor solution. Distilled water is added to the 250-ml. mark, and the solution is thoroughly mixed.

Ten to 15 ml. of the final solution are transferred to the measuring cell containing the mercury-pool electrode. The dropping mercury electrode is adjusted so that the lower end is approximately 0.6 cm. above the surface of the mercury pool. The cell is purged with oxygen-free nitrogen for 3 to 5 minutes at 200 to 250 ml. per minute. After the electrical leads are connected to the measuring-cell electrodes, current to the instrument is turned on (left-hand knob) and the galvanometer is checked for zero. A fixed pattern of operations involving the successive depression of the four voltage-applying push buttons, followed in each case by adjustment of the associated potentiometer to produce an average zero on the galvanometer, leads to the last such adjustment, with the calibrated dial, which yields the final result in milliliters of tetraethyllead per gallon.
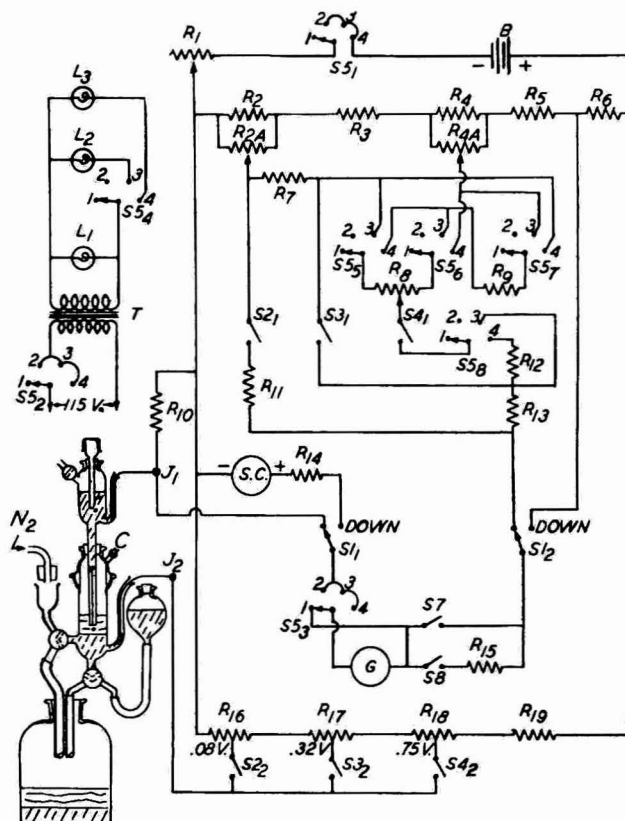

Figure 3. Electrical Wiring Diagram of Direct-Reading Polarograph

An experienced analyst can perform sixteen complete tetraethyllead analyses in an 8-hour working day when using the new polarographic instrument and the A.S.T.M. extraction procedure, with a time allowance for making reports and cleaning equipment. In order to do this, a set of four extraction vessels arranged for simultaneous operation is required. Thus 0.5 hour of consumed time is required for one complete tetraethyllead analysis. In 10 minutes after completion of the extraction, the result for the tetraethyllead content of a single sample could be available. Under optimum conditions an elapsed time of 1.5 hours is needed for an analysis. By way of comparison, the A.S.T.M. gravimetric procedure requires an elapsed time of 7.5 hours, of which 72 minutes are consumed time.

Calibration. Calibration of the new polarograph is carried out using the standard lead solution previously described.

A portion of this equivalent to the desired concentration of tetraethyllead per gallon is added to a 250-ml. graduated cylinder; 25 ml. of concentrated hydrochloric acid, 5.00 ml. of pilot-ion solution, and 5 ml. of maxima suppressor are added. After dilution to 250 ml., and mixing, a portion of the solution is placed in

Table II. Repeatability Comparison of Polarographic and A.S.T.M.

(Ml. of TEL per gallon of gasoline)

Sample No.		Polarographic	A.S.T.M.	Difference, Polarographic − A.S.T.M.
1		2.07	2.07	−0.01
		2.06	2.08	
		2.08	2.09	
	Av.	2.07	2.08	
2		2.45	2.47	−0.03
		2.45	2.48	
		2.44	2.48	
	Av.	2.45	2.48	
3		2.50	2.49	+0.03
		2.51	2.49	
		2.54	2.49	
	Av.	2.52	2.49	
4		2.82	2.84	−0.02
		2.79	2.83	
		2.81	2.83	
	Av.	2.81	2.83	

the cell chamber and the polarographic measurement is made as described under "Procedure." In the original instrument calibration, points were set up at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 ml. of tetraethyllead per gallon, the dial being marked for the equivalent tetraethyllead value following each final instrument adjustment.

PRECISION AND ACCURACY

In order to obtain a measure of the accuracy of the over-all method including tetraethyllead decomposition and extraction and subsequent polarographic measurement, a series of test samples was prepared for analysis. These samples were made up by the addition of known amounts of tetraethyllead to "iso-octane" (2,2,4-trimethylpentane) to cover the range of 0.5 to 8.0 ml. of tetraethyllead per gallon. Table I shows the results obtained for this series of complete analyses performed in quadruplicate by a single analyst.

These data indicate that the over-all accuracy of the polarographic method is within 0.02 ml. of tetraethyllead per gallon in the range of 0.5 to 3.5 ml., and within 0.03 ml. of tetraethyllead per gallon in the range of 3.5 to 7.9 ml. The quadruplicate analyses also show repeatability within 0.02 ml. of the mean for the entire range 0.5 to 7.9 ml. of tetraethyllead per gallon.

In order to compare the repeatability of the polarographic method with that of the A.S.T.M. method for actual gasoline analyses, complete triplicate analyses of a number of different gasoline samples have been performed by both methods. The polarographic and A.S.T.M. values shown in Table II were determined by different analysts using a different extraction apparatus for each method.

The data in Table II confirm the results given in Table I, showing that the repeatability of the new polarographic method for analyses of tetraethyllead in gasoline is within 0.02 ml. of tetraethyllead per gallon in the range of 2 to 3 ml. of tetraethyllead per gallon. The comparative analyses show that the polarographic method is in agreement with the A.S.T.M. method within 0.03 ml. of tetraethyllead per gallon in the range of 1 to 3 ml. of tetraethyllead per gallon, which deviation is within the probable accuracy of the two methods. The above analyses and those re-

ported hereinafter were performed upon typical refinery gasoline production from a variety of sources. Some represent fresh manufacture, whereas others had aged by a number of months of storage before they were tested. Hence, the reproducibility shown represents that attainable in the presence of unsaturates, peroxides, etc., which may be present in motor fuel.

Comparative analyses performed in a routine manner with the polarographic and A.S.T.M. methods by different analysts and extraction apparatus are given in Table III for a variety of premium and regular gasolines made by a number of different refiners. The polarographic analyses were determined in duplicate, while single analyses were made by the A.S.T.M. method.

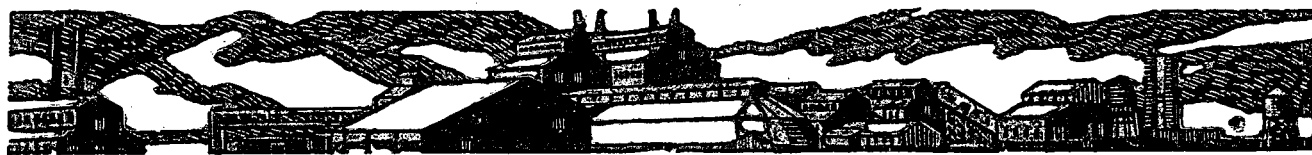**Table III. Routine Determination of Tetraethyllead in Gasoline**

(Ml. of TEL per gallon of gasoline)

Sample No.	Polarographic 1st	Polarographic 2nd	Polarographic Av.	A.S.T.M. (Single)	Difference, Polarographic − A.S.T.M.
1	1.13	1.14	1.13	1.13	0.00
2	1.23	1.23	1.23	1.27	−0.04
3	1.49	1.51	1.50	1.48	+0.02
4	1.52	1.53	1.52	1.49	+0.03
5	1.59	1.61	1.60	1.57	+0.03
6	1.63	1.65	1.64	1.64	0.00
7	1.65	1.65	1.65	1.64	+0.01
8	1.67	1.68	1.67	1.63	+0.04
9	1.71	1.73	1.72	1.73	−0.01
10	1.76	1.77	1.77	1.80	−0.03
11	1.80	1.84	1.82	1.85	−0.03
12	1.82	1.83	1.82	1.80	+0.02
13	1.83	1.84	1.84	1.85	−0.01
14	1.83	1.85	1.84	1.86	−0.02
15	1.83	1.88	1.85	1.83	+0.02
16	1.92	1.93	1.93	1.95	−0.02
17	1.93	1.93	1.93	1.99	−0.06
18	1.99	1.99	1.99	2.02	−0.03
19	2.12	2.13	2.13	2.15	−0.02
20	2.16	2.19	2.17	2.10	+0.07
21	2.21	2.22	2.21	2.21	0.00
22	2.31	2.33	2.32	2.33	−0.01
23	2.41	2.42	2.41	2.36	+0.05
24	2.51	2.53	2.52	2.52	0.00
25	2.51	2.53	2.52	2.56	−0.04
26	2.54	2.58	2.56	2.58	−0.02
27	2.64	2.67	2.65	2.63	+0.02
28	2.72	2.75	2.73	2.72	+0.01
29	2.83	2.83	2.83	2.81	+0.02
30	2.89	2.91	2.90	2.90	0.00
31	2.91	2.93	2.92	2.97	−0.05
32	3.13	3.14	3.14	3.16	−0.02
				Av.	0.023

The data in Table III show that in routine use the new polarographic method is in satisfactory agreement with the A.S.T.M. method, the arithmetical average of the differences between the analyses of thirty-two different gasolines performed with the two methods being only slightly greater than 0.02 ml. of tetraethyllead per gallon.

LITERATURE CITED

(1) Am. Soc. Testing Materials, Philadelphia, Pa., "A.S.T.M. Standards on Petroleum Products and Lubricants," pp. 934–6, Designation D 526–48T, 1949.
(2) Borup, R., and Levin, H., Am. Soc. Testing Materials Proc., 47, 1010 (1947).
(3) Calingaert, G., and Gambrill, C. M., IND. ENG. CHEM., ANAL. ED., 11, 324 (1939).
(4) Frediani, H. A., and Bass, L. A., Oil Gas J., 39, No. 20, 51 (1940).
(5) Kahan, G. J., IND. ENG. CHEM., ANAL. ED., 14, 549 (1942).
(6) Mueller, E. F., Ibid., 12, 171 (1940).

Determination of Tetraethyllead in Gasoline by X-Ray Absorptiometry

GEORGE CALINGAERT[1], F. W. LAMB, H. L. MILLER[2], AND G. E. NOAKES[3]

Research Laboratories, Ethyl Corporation, Detroit, Mich.

The results obtained in an investigation of x-ray absorptiometry as a method for the determination of tetraethyllead in gasoline are described. The sensitivity of the absorbance measurements to voltage fluctuations made it imperative to be able to adjust and maintain the primary voltage constant to ±0.10 volt. The refinements necessary to obtain this voltage constancy are given, as well as other modifications made to the General Electric x-ray photometer which resulted in improved performance. The sensitivity of the absorbance measurements of gasoline samples to voltage fluctuations was decreased by a factor of 10 by the introduction of a polystyrene block in the reference beam. The calibration data obtained for tetraethyllead in gasoline showed the method to be sensitive to 0.01 ml. of tetraethyllead per gallon. The precision of the method is ±0.01 ml. of tetraethyllead per gallon. In the analysis of samples for which the respective unleaded base stocks are available, the results are accurate to ±0.01 ml. of tetraethyllead per gallon. The analysis of 68 samples for which the respective base stocks were not available showed the results to be accurate to ±0.05 ml. of tetraethyllead per gallon. The varying percentage of sulfur in gasoline was found to be the chief obstacle to a more accurate determination of tetraethyllead by x-ray absorptiometry. Possible methods for eliminating problems due to varying sulfur and halogen concentrations in gasoline are discussed. All apparent anomalies encountered in absorbance measurements are accounted for by scattering and filtering effects.

THE Research Laboratories of the Ethyl Corporation have been interested for many years in the possible application of x-ray absorptiometry to the determination of tetraethyllead (TEL) in gasoline. Based on the principles of x-ray absorption, it was anticipated that the increased absorbancy due to the presence of a heavy element such as lead in a medium of light elements such as carbon and hydrogen would be appreciable. Furthermore, it was thought that such a rapid, nondestructive physical means of analysis would be of considerable value in the routine testing of gasolines. Early experiments indicated that the method could be used but that it was not practical with the equipment then available. During the past few years several new developments in the field of radiation detectors and electronics have made it possible greatly to improve the sensitivity and stability of instruments for use in x-ray absorption studies. This improvement in instrumentation has resulted in a renewed interest in the application of x-ray absorptiometry to the determination of tetraethyllead in gasoline, sulfur in petroleum products, and halogens in plastics.

Liebhafsky (6) has given a review of the fundamental principles of x-ray absorptiometry, its promising applications, and a description of the General Electric x-ray photometer. Additional information is given in well-known texts (2, 3, 9) on the subject and by Aborn and Brown (1), Liebhafsky et al. (7, 13), and Sullivan and Friedman (10). This paper is concerned chiefly with the experience of and the results obtained by the Ethyl Laboratories in developing a method for the determination of tetraethyllead in gasoline by use of this instrument.

The first published work on this problem was by Aborn and Brown (1) in 1929. The results obtained, however, were limited in sensitivity because of the detecting systems available at that time. Gross and Staab (4) of the German Aeronautical Research Staff reported in 1941 on an x-ray absorption method for the

determination of tetraethyllead in gasoline. From a theoretical standpoint their analysis of the problem, including the possible interference of sulfur, was good; however, their apparatus was not adequate for the accuracy desired. Sullivan and Friedman (10) of the Naval Research Laboratories reported in 1946 on the use of a Geiger-counter receiver for the determination of tetraethyllead in gasoline by x-ray absorption. Their equipment consisted of a molybdenum-target tube operated at 17 kv. (corresponding to an effective wave length of approximately 0.94 A., which has the advantage of being close to the L_{III} absorption edge of lead), a 15-cm. cylindrical brass absorption cell with thin aluminum windows, and a specially developed 80% efficient Geiger-counter tube for measuring x-ray intensity. The method proved to be sensitive to 0.005 ml. of tetraethyllead per gallon, and the standard deviation was reported to be 0.05 ml. tetraethyllead per gallon. The results reported by Liebhafsky (13) in 1949 on tetraethyllead in gasolines for which the unleaded base stocks were known and available also showed that the method has considerable promise. A paper was presented by Vollmar (12) of the Standard Oil Company of California before the Division of Petroleum Chemistry at the 1949 spring meeting of the AMERICAN CHEMICAL SOCIETY on the results obtained in the determination of tetraethyllead in gasoline using the General Electric x-ray photometer. His results and conclusions closely parallel those of these laboratories—namely, best results are obtained when the original unleaded base stocks are available for comparison with the samples, and the presence of varying amounts of sulfur in the gasoline is the chief source of error.

As noted by Vollmar, the problem is not as simple as it appears, for there are numerous types of gasoline base stocks, and the tetraethyllead may be added either alone (as in research studies) or as two standard commercial antiknock mixtures. One mixture contains tetraethyllead with a definite proportion of ethylene dibromide and ethylene dichloride, and the other contains tetraethyllead with a definite proportion of ethylene dibromide. Hereinafter these two commercial antiknock mixtures are referred to as 62 Mix and 1-T Mix, respectively. Because x-ray absorption is an additive function of all the elements present in

the sample, a single x-ray absorbance measurement of a gasoline sample could not be expected to differentiate the various anti-knock fluids or to correct for the differences in absorbance due to the various base stocks. Preliminary work with gasoline samples indicated that some independent means of correcting for the absorbance of the various base stocks is needed, for one has no knowledge of the respective unleaded base stocks of the majority of samples of gasolines submitted for routine lead analysis.

The specific problems of this investigation were:

To determine the variation in absorbance due to various base stocks and to investigate methods for minimizing or correcting for this variation.

To establish the relationship between absorbance and milliliters of tetraethyllead per gallon of hydrocarbon as tetraethyllead, 1-T, or 62 Mix.

To investigate the possibility of determining the type of anti-knock mixture by obtaining absorbance readings at more than one selected effective wave length.

To determine the precision and sensitivity of the method.

To determine the over-all accuracy of the method as applied to routine gasoline submitted for chemical analysis.

MODIFICATIONS TO INSTRUMENT

Briefly, the General Electric x-ray photometer is a double-beam null-type instrument in which a comparison of the absorbance in the two beams is made 30 times a second by means of an electronic peak comparator system. The source of radiation (polychromatic) is a tungsten-target tube operated at voltages up to 46 kv., and the receiver comprises a sensitive phosphor and a multiplier phototube. A detailed description of the instrument and its principle of operation is given by Rich and Michel (8).

In preliminary work with this instrument it was found necessary to make several modifications to attain the required sensitivity, reproducibility, and stability for the development of a satisfactory analytical method. A few minor modifications were made to provide for alignment of the various units (absorption cell, alternate chopping device, diaphragm septum, and receiving system), so that their two horizontal axes would be at right angles to the central x-ray beam. The supports for these parts were also reinforced to maintain their proper alignment once they were determined. A diagram showing the relative positions of these units is given by Rich and Michel (8, Figure 1). In addition, the following major changes were made:

Installation of Sorensen Voltage Regulator and Study of Voltage Characteristics. The instability of the balance point was observed to be greater when a hydrocarbon was balanced against aluminum than when aluminum was balanced against aluminum. It was therefore reasoned that the difficulty was

Figure 1. Wave Forms of Input and Output Voltages, as Obtained with Oscilloscope

A. Without voltage regulator
B. With Sorensen 2-kv.-amp. voltage regulator
C. With Sorensen 10-kv.-amp. voltage regulator
1. Input voltage
2. Output voltage from photomultiplier tube

due to fluctuations in the line voltage, because this would show up as variations in effective wave length.

A series of absorbance measurements was made to demonstrate the sensitivity of the measurements to variations in primary voltage. Using 200 ml. of "iso-octane" (2,2,4-trimethylpentane) in the sample beam and balancing the absorbance with varying thicknesses of aluminum, measurements were obtained at primary voltages of 95, 100, and 105. From these measurements it was calculated that a fluctuation of 1.0 volt produced a difference in absorbance of 1.7 mils of aluminum. This is roughly equivalent to 0.10 to 0.15 ml. of tetraethyllead per gallon. Because the voltage fluctuations were easily of the order of 1 to 2 volts, the need for voltage regulation was indicated.

Table I. Equivalent R.M.S. Voltages to Give Same X-Ray Peak Intensities with Voltage Regulator In (VRI) and Out (VRO)

Input Voltage (Equivalent R.M.S. Voltage)	Condition of Test	Al, Mils	Kvp.	Effective λ, A.
100.0	VRI	367.5	31.2	0.51
86.0	VRO	367.5	31.2[a]	0.51[a]
75.0	VRI	178.6	24.2	0.67
66.5	VRO	178.6	24.2	0.67
50.0	VRI	55.3	16.9	0.95
47.0	VRO	55.3	16.9	0.95

[a] Peak voltage (kvp.) and its corresponding effective wave length for input voltage with voltage regulator out are obtained from Figure 2, which shows relationship between input voltage, peak voltage, and effective wave length for instrument. Data for Figure 2 were supplied by General Electric Company.
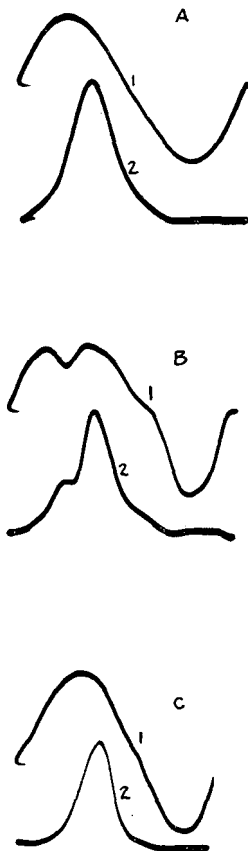
A Sorensen 2-kv.-amp. voltage regulator model 1750-S was installed and, as a result, the fluctuations in input voltages were greatly reduced. At this point, however, the inadequacy of the voltmeter and the means of voltage adjustment was recognized. The input voltage could be adjusted only in steps of 0.5 volt and the voltmeter was good only to 0.5 volt. A precision voltmeter which could be read accurately to 0.05 volt and an additional rheostat to give a fine adjustment within each 0.5-volt step were installed. With these refinements it was a simple matter to maintain the input voltage within limits of ±0.1 volt of the desired level for several hours.

A repetition of the previous "sensitivity to voltage fluctuation" test showed that with the voltage regulator connected, a fluctuation of 1 volt produced a difference in absorbance of 2.9 mils of aluminum. It was also noted that the absorbance readings for an input voltage of 100 were higher for hydrocarbons without the voltage regulator than with it. Fortunately, the difference in absorbance due to added increments of lead was the same under both conditions. Thus, the sensitivity for lead remains unchanged, although the peak voltage is lower and the consequent effective wave length is higher, as explained below.

It was desired to obtain a measure of the peak potential and the consequent effective wave length when using the voltage regulator. This was accomplished by determining the equivalent root mean square voltages both with and without the voltage regulator to give the same x-ray peak intensities. To accomplish this, the right-hand beam was blocked with lead sheet and the left-hand beam was used as a single-beam instrument. The following measurements were made: (1) with the voltage regulator in the circuit and an input voltage of 100, the thickness of aluminum to give a peak intensity reading of 90 on the amplification level was determined, (2) with the voltage regulator out and the same thickness of aluminum as determined in (1), the input voltage to give a peak intensity reading of 90 was determined. The equivalent root mean square voltages were obtained by steps (1) and (2) at 100, 75, and 50 input volts (Table I).

With the voltage regulator out and an input voltage of 100 the effective wave length is approximately 0.44 A., whereas with the voltage regulator in and an input voltage of 100 the effective wave length is 0.51 A. From Figures 5 and 6 it is seen that the

absorbance in mils of aluminum for hydrocarbons is appreciably higher at 0.44 A., whereas the absorbance for lead in mils of aluminum is not markedly different over this range of effective wave lengths. Thus, the lower absorbance values obtained for hydrocarbons when using the voltage regulator is explained. Likewise, the fact that the sensitivity for lead is the same both with and without the voltage regulator becomes evident.
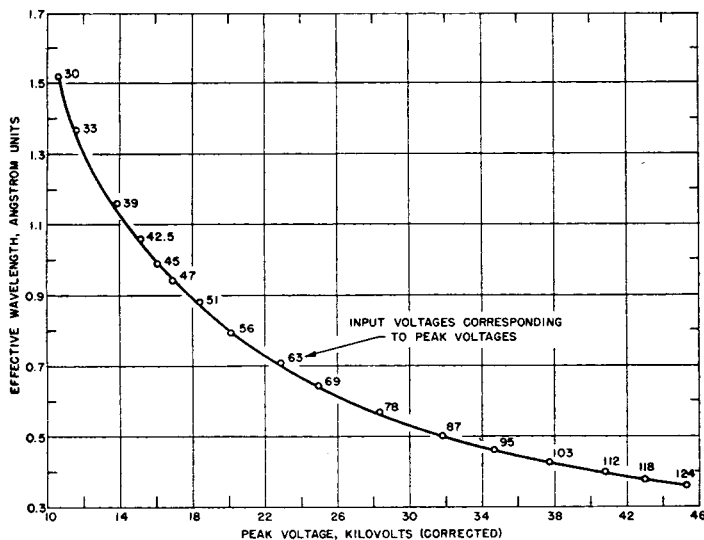

Figure 2. Calculated "Effective" Wave Lengths for Continuous Radiation from Tungsten-Target Tube

The wave forms of the input voltage and the output voltage from the photomultiplier tube both with and without the voltage regulator were observed with a cathode-ray oscilloscope. Simultaneous photographs were taken of the wave forms of the input and output voltages by using two oscilloscopes and feeding the output of the two separate oscilloscopes to a two-gun cathode-ray tube in order to obtain the composite pictures shown in Figure 1. The curves obtained without the voltage regulator (Figure 1, A) show very little distortion, whereas the curves obtained with the 2-kv.-amp. regulator (Figure 1, B) show obvious distortion.

It is evident from Figure 1, B, that the Sorensen 2-kv.-amp. voltage regulator does not have the desired capacity for the load at the peak voltage of the x-ray tube. At the time of completion of the present work a Sorensen 10-kv.-amp. voltage regulator Model 10,000-2 was available for test. The wave forms obtained with the 10-kv.-amp. regulator (Figure 1, C) show marked improvement over the wave forms obtained with the 2-kv.-amp. regulator. Although the balance needle showed troublesome fluctuations during this test, it is believed that by applying the proper load, further improvement in the performance of the instrument could be obtained by use of the 10-kv.-amp. voltage regulator.

Installation of Silver Commutator with Silver-Graphite Brushes. After the aforementioned modifications were made to ensure voltage stability, it was observed that for an absorbance equivalent to 50 mils of aluminum the conditions of balance were satisfactory as regards sensitivity and reproducibility. At higher absorbance levels, however, the balance point became less sensitive and less reproducible. With 200 to 300 mils of aluminum, which is in the absorbance range required for the determination of tetraethyllead in gasoline, the sensitivity and reproducibility of the balance points were still far from satisfactory. Also, a steady drift of the balance point during continued operation was noted. It was believed the copper oxide rectifiers in the phase-sensing

detector bridge circuit were causing the difficulty and these were replaced with a new detecting system employing a synchronously driven silver commutator in conjunction with stationary silver-graphite brushes. At the same time a graphite brush was installed to ground the rotating shaft of the alternating chopper. In addition, provision was made for periodic cleaning of the contact surface of the commutator.

With this new detecting system the instrument is almost entirely free from temperature drift and is definitely improved in the sensitivity and reproducibility of the balance point at the absorbance level of 200 to 300 mils of aluminum. Furthermore, the instrument can now be phased and balanced much more easily.

Resulting Performance of Instrument. The over-all performance of the modified instrument showed considerable improvement. The input voltage could be set at a desired voltage with an accuracy of ±0.05 volt and it would remain constant to ±0.10 volt for 0.5 to 3 hours. In fact, it was found that after a warm-up period of 15 to 30 minutes with the x-ray tube operating, the input voltage would register within 0.10 volt of the voltage used the previous day. Observation of this variation in input voltage became one of the daily routine checks of instrument performance. Daily checks were also made of the absorbance readings of a 48-mil aluminum block and a polystyrene block having an absorbance equivalent to 125 mils of aluminum. The absorbance readings of aluminum versus aluminum were reproducible to ±0.1 mil and those of hydrocarbons versus aluminum to ±0.4 mil for periods of several weeks.

A check of the sensitivity of the absorbance readings was also made each day the instrument was used. The balance meter (30-0-30 microammeter) has 20 divisions on each side of the zero. After balancing the absorbance of the two beams for the 48-mil aluminum block, measurements were made to determine how many mils of aluminum were required to throw the balance needle 20 divisions to the right or left. This measured sensitivity expressed as meter divisions per 0.1 mil of aluminum varied from 1.6 to 3.1 during a period of several weeks. When the sensitivity, measured in this way, fell below 1.5 the instrument performance was considered to be too insensitive for satisfactory use. This condition could usually be corrected by cleaning the contact surfaces of the phase-sensing system.

DEVELOPMENT OF METHOD FOR DETERMINATION OF TETRAETHYLLEAD IN GASOLINE

Preliminary work showed that the sensitivity for tetraethyllead is greater at the higher input voltages (lower effective wave length). The cause is readily seen from Figure 5, where the ratios of the absorption coefficients of tetraethyllead to those of aluminum are shown as a function of wave length. For this reason an input voltage of 100 was used throughout the work. It was soon recognized that the more similar the absorbing

Table II. Sensitivity to Voltage Fluctuation

Material in Sample Beam	Sensitivity, Mils Al/0.1 Volt. in Input Voltage Range of 95-105
Without Polystyrene Block in Reference Cell	
Iso-octane	0.25
Toluene	0.31
Base stock A	0.30
Base stock A + 5.0 ml. TEL/gal.	0.30
With Polystyrene Block in Reference Cell	
Iso-octane	0.031
Base stock A	0.023
Base stock B	0.023
Benzene	0.024
Aviation 73	0.026

elements in the two beams, the less sensitive the absorbance measurements were to fluctuations in voltage. Thus, absorbance readings of hydrocarbon versus aluminum were more subject to voltage variations than were readings of aluminum versus aluminum. The use of a hydrocarbon standard absorber in the reference beam was indicated.

Use of Hydrocarbon Block in Reference Beam. A polystyrene block having an absorbance slightly less than that of 200 ml. of iso-octane was machined to fit the left-hand side of the absorption cell. The use of this standard absorber in the reference beam decreased the sensitivity to voltage fluctuations of the absorbance readings of gasoline samples by a factor of 10, as may be seen from Table II.
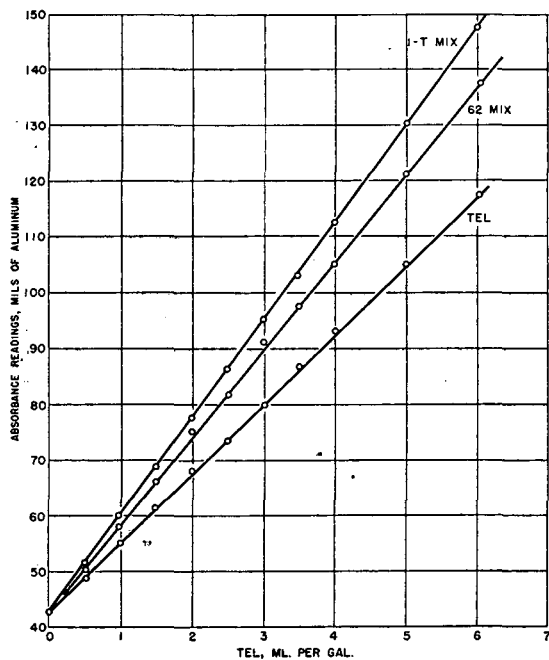
Figure 3. Calibration Data for Tetraethyllead in Base Stock A

Constant volume 200 ml.

The reproducibility of all absorbance measurements involving hydrocarbons in the right-hand beam was greatly improved by the use of the polystyrene block in the left-hand beam, and it was therefore used for all subsequent measurements.

An investigation of two general tetraethyllead procedures was made: (1) using constant-volume samples of 200 ml. and (2) using constant-weight samples of 150 grams. The standardized conditions used for all absorbance measurements on standards and samples, unless otherwise stated, were as follows: (1) input voltage (Sorensen voltage regulator connected), 100 volts; (2) emission, 10 ma.; (3) amplification level, 60 microamperes; and (4) polystyrene block in the reference beam.

Constant-Volume Samples. Standard samples were prepared in iso-octane and in unleaded base stock A, containing 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, and 6.0 ml. of tetraethyllead per gallon (at 20.0° C.) added as tetraethyllead alone, as 62 Mix, and as 1-T Mix. Absorbance measurements were obtained on 200-ml. samples introduced in the sample cell by means of an automatic gasoline pipet. The tetraethyllead concentrations were corrected for the temperature at which the aliquots and absorbance readings were taken. Graphs of the corrected concentrations in milliliters against absorbance in mils of aluminum were straight lines, the absorbance intercept depending on the base stock and the slope depending on the antiknock mixture. The sensitivities for tetraethyllead and the two standard mixtures are given in Table III. These data are also shown graphi-

cally in Figure 3 for the standards in base stock A. For the standards in iso-octane the same family of lines was found to intersect at the absorbance value for iso-octane (approximately 26.5 mils of aluminum).

Absorbance measurements obtained on iso-octane, toluene, benzene, and the unleaded base stocks showed a nearly linear relationship between density and absorbance at constant volume for the various hydrocarbons. This relationship is to be expected for binary mixtures of hydrogen and carbon, because their mass absorption coefficients are very nearly the same for the wavelength range used (see Figures 5 and 6 for a comparison of the absorption coefficients of iso-octane and benzene as a function of wave length). A summary of the density-absorbance data obtained on a number of unleaded base stocks is shown in Figure 4. It will be noted that three parallel straight lines are indicated; the upper one (I) passes through Aircraft 73 and samples of various shipments of base stock A, the middle one (II) through the pure hydrocarbons, iso-octane and benzene, and the lower one (III) through unleaded base stocks B and C. From these results it is evident that a correction for the absorbance of the unleaded base stock based on its density may be in error by as much as ±0.06 ml. of tetraethyllead per gallon.

Table III. Calibration Data for Tetraethyllead in Gasoline

TEL as	Constant Volume of 200 Ml., Mils Al/Ml. TEL/Gal.	Constant Weight of 150 Grams, Mils Al/G. TEL/Kg.
TEL	12.0	21.4
62 Mix	15.5	27.5
1-T Mix	17.5	31.3

Using the calibration data given in Table III and the relationship of density to absorbance given in Figure 4, a number of routine gasoline samples were analyzed. The following slope-intercept equation was used to calculate the results in milliliters of tetraethyllead per gallon, which were then converted to the corresponding concentrations at 15.5° C. for comparison with the results obtained by chemical analysis.

$$y = mx + b \qquad (1)$$

where y = the absorbance of the sample in mils of aluminum
  m = the slope (mils of aluminum equivalent to 1 ml. of TEL per gallon from Table III)
  b = the absorbance of the unleaded base stock in mils of aluminum obtained from Figure 4
  x = the ml. of TEL per gallon of the sample

For an analysis of tetraethyllead in gasoline by the constant-volume method the following steps are required:

1. Introduce a 200-ml. sample of gasoline into the absorption cell and measure its absorbance, y.
2. Obtain the density at the temperature of the absorbance reading and convert to the corresponding b value from Figure 4.
3. Using the proper value for m from Table III calculate x and correct to milliliters of tetraethyllead per gallon at 15.5° C.

A comparison of the results obtained by chemical analysis and x-ray absorption at constant volume on 68 samples showed an average difference of 0.05 ml. of tetraethyllead per gallon with a difference spread of +0.12 to −0.11 ml. of tetraethyllead per gallon. The over-all average deviation of the x-ray absorption determinations was 0.01 ml. of tetraethyllead per gallon. The concentrations of the samples ranged from 0.00 to 4.12 ml. of tetraethyllead per gallon as 62 Mix. In all subsequent sample analyses the tetraethyllead is present as 62 Mix unless otherwise specified.

In the course of analyzing additional gasoline samples submitted for chemical analysis, unusually low results were obtained on two series of leaded gasolines. Investigation showed that both series were prepared from base stocks obtained from the

same supplier: one from "regular" base and the other from "premium" base. A sample of the unleaded regular base stock was available for absorbance measurement; the absorbance reading for this sample (referred to as base stock C) falls on curve III of Figure 4 and is therefore similar in absorbance properties to base stock B. The tetraethyllead results for the six leaded gasolines as calculated initially using b values from Figure 4, I, and the more nearly correct values using b from Figure 4, III, are given in Table IV.
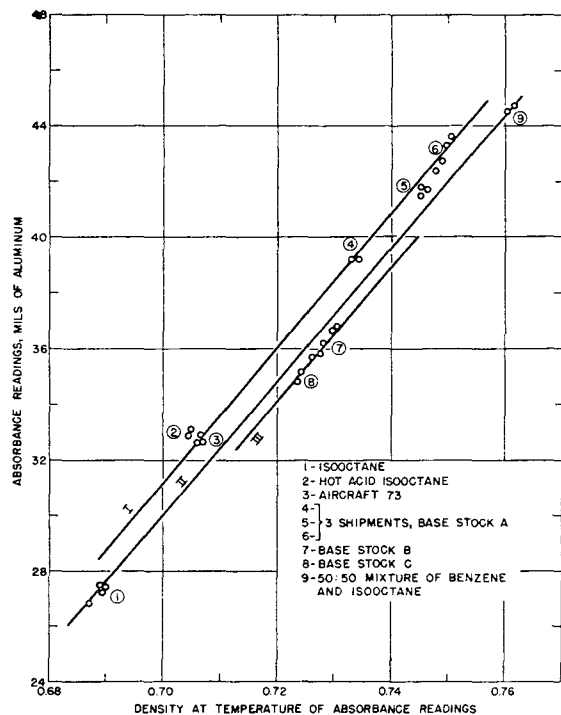
Figure 4. Relationship of Density and Absorbance

200-ml. samples of hydrocarbons

Thus it is seen that the analysis of tetraethyllead in the run of the mill gasoline samples is subject to error because of the possible variations in base stock absorbance. Experience with additional base stocks might well reveal greater variations than those represented in Figure 4.

Constant-Weight Samples. It was initially felt that errors due to geometric considerations would be introduced by adopting constant-weight samples, because this would mean absorbance measurements at various depths of solution, depending on the density of the solution. However, a test of the absorbance of 150 grams of benzene at different temperatures gave very nearly constant results. At 8° to 13° C. the absorbance reading was 42.0; at 18° C., 42.0; at 25° C., 41.9; and at 40° C., 41.9. This constancy of absorbance reading with a density variation and subsequent depth variation of 3.7% indicated that no appreciable error would be introduced by variations in density of this order. Calibration data in terms of mils of aluminum per gram of tetraethyllead per kilogram were obtained on 150-gram samples of the same standards as used for the constant-volume method. Linear relationships were obtained for absorbance versus gram of tetraethyllead per kilogram of sample. The calibration data obtained are shown in Table III.

The absorbance data for tetraethyllead in iso-octane and base stock A determine two parallel straight lines, the one for base stock A being at an absorbance level of approximately 2.0 mils of aluminum above that for the iso-octane standards. This difference is comparable in amount with the variation (as much

as 2.5 mils of aluminum) found in the absorbance readings of 150-gram samples of various unleaded base stocks. The absorbance data on 150-gram samples of hydrocarbons and unleaded base stocks are given in Table V.

The steps involved in the determination of tetraethyllead by the constant-weight method are:

1. Weigh a 150-gram sample to 0.1 gram and measure its absorbance, y.
2. Using the proper value for m from Table III and for b from Table V, calculate x, the grams of tetraethyllead per kilogram of hydrocarbon, using Equation 1 and substituting units of weight for units of volume.
3. Obtain density corrected to 15.5° C. and convert x obtained in (2) to milliliters of tetraethyllead per gallon at 15.5° C. by the following equation:

$$\text{Ml. of TEL/gal.} = \frac{\text{g. of TEL/kg.} \times 3.7854 \times \rho}{1.65} \quad (2)$$

where ρ is the density of the sample at 15.5° C. and 1.65 is the number of grams of tetraethyllead per defined "ml. of tetraethyllead."

While the same measurements, absorbance and density, are required by both methods, analysis by the constant-weight method has the advantage that the samples and subsequent absorbance measurements are independent of temperature variation. Also, the standards may be prepared directly on a weight basis. The constant-volume method has the advantage of greater speed and is free from errors introduced by scattering as discussed in the section on Apparent Anomalies in Absorbance Measurements.

A comparison of the results obtained by the two absorption methods for twelve samples is given in Table VI.

From Table VI it is seen that both absorption methods give about the same results and therefore have about the same errors.

Table IV. Analysis of Base Stock C Samples

	Ml. of TEL/Gal. at 15.5° C.			Δ
	X-Ray Absorption Using b from:			Chem-X-Ray
Sample Description	Fig. 4, I	Fig. 4, III	Chemical	(Fig. 4, I)
Regular, unleaded	−0.12	0.00	0.00	−0.12
Regular	+0.39	0.50	0.49	−0.10
Regular	+1.42	1.54	1.49	−0.07
Regular	+2.80	2.96	2.98	−0.18
Premium	+0.40	0.46	0.47	−0.07
Premium	+1.37	1.43	1.46	−0.09
Premium	+2.83	2.90	2.94	−0.11

Table V. Absorbance of Unleaded Base Stocks

(150-gram samples)

Base Stock	Absorbance, Mils Al (in Addition to Polystyrene Block)	Density at 15.5° C.
Iso-octane	41.2	0.701
Benzene	42.0	0.883
Toluene	41.6	0.870
Base stock B	42.0	0.738
Aircraft 73	43.0–43.5	0.717 ± 0.01
Base stock A	42.8–43.6	0.745 ± 0.01
Hot acid iso-octane	43.2	0.712

Table VI. Comparison of Results by Constant-Volume and Constant-Weight Methods

	Ml. of TEL/Gal. at 15.5° C. by		
Sample No.	Constant-vol.	Constant-wt.	Δ
1	2.44	2.49	−0.05
2	0.77	0.79	−0.02
3	0.79	0.79	0.00
4	2.91	2.91	0.00
5	1.02	1.03	−0.01
6	1.51	1.55	−0.04
7[a]	1.40	1.38	+0.02
8	0.40	0.41	−0.01
9	0.02	0.04	−0.02
10	2.88	2.90	−0.02
11	3.15	3.12	+0.03
12	2.98	3.00	−0.02

[a] Constant-weight data calculated using b = 42.0 for sample 7 and b = 43.5 for other samples.

Table VII. Absorbance Measurements of Synthetic Hydrocarbon Mixtures Containing Sulfur, Nitrogen, and Oxygen

Description of Sample	Element Added, Wt. %	Absorbance Readings at Input Voltages of:					Element Equivalent to 1 Mil Al at 100 Volts, Wt. %
		90	95	100	105	110	
125 g. iso-octane + 25 g. C₂H₅OH	5.66 oxygen	46.4	48.2	49.2	50.9	52.8	
Pure iso-octane		37.6	38.9	40.3	42.0	44.0	0.64
		Δ' 8.8	9.3	8.9	8.9	8.8	
125 g. iso-octane + 25 g. ethyl-m-toluidine	1.66 nitrogen	39.2	40.4	42.2	44.1	45.6	0.87
		Δ' 1.6	1.5	1.9	2.1	1.6	
149 g. iso-octane + 1 g. thiophene	0.25 sulfur	44.8	46.2	47.5	49.2	51.3	0.035
		Δ' 7.2	7.3	7.2	7.2	7.3	

Δ' in each case is difference in absorbance between synthetic sample and pure iso-octane.

This is to be expected from a consideration of the absorbance data (Figure 4 and Table V) on the unleaded base stocks by the two methods.

Effect of Interfering Elements, Particularly Sulfur. It was thought that the difference in absorbance of the base stocks might be due to interference from other compounds containing lead, nitrogen, sulfur, or oxygen. Spectrographic analysis indicated the absence of lead in the various unleaded base stocks. Absorbance measurements made on unleaded iso-octane, and base stocks A and B, both before and after evaporation to one half their original volumes, definitely indicated that base stock A and base stock B (to a lesser extent) contained a material less volatile than the evaporated fraction.

Absorbance measurements of synthetic mixtures of iso-octane with ethyl alcohol, with thiophene, and with ethyl-m-toluidine indicated that a sulfur-containing compound is the most interfering substance. The absorbance readings and compositions of synthetic mixtures are given in Table VII. The data show that as little as 0.06% sulfur would account for the difference in absorbance between iso-octane and base stock A. A much larger percentage of nitrogen or oxygen would be required to account for this difference.

From these data it was calculated that the presence of 0.10% sulfur in a gasoline, if no correction were made, would result in a tetraethyllead determination high by 0.24, 0.18, or 0.16 ml. per gallon when the tetraethyllead was present as tetraethyllead, 62 Mix, or 1-T Mix, respectively. These experimental figures are in close agreement with the values calculated from the mass absorption coefficients given in the literature, from which 0.10% sulfur is equivalent to 0.185 ml. of tetraethyllead (as 62 Mix) per gallon for the wave-length range 0.417 to 0.710 A. (Table XII). Thus it is seen that the normal variation in weight per cent sulfur (0.03 to 0.15) of present gasolines results in a practical limitation of about 0.11 ml. of tetraethyllead per gallon to the accuracy which may be attained by x-ray absorption methods, if standardization data based on base stocks containing 0.09% sulfur were used. However, some Pacific coast gasolines may contain as much as 0.3% sulfur and would therefore require some special calibration to correct for the high sulfur content. In cases where the sulfur content is known corrections can be applied on the basis of the aforementioned relationship. In aviation gasolines, where the sulfur content is low and varies but little (0.004 to 0.020%), calibration data allowing for a sulfur content of 0.012% should give results accurate to ±0.03 ml. of tetraethyllead per gallon, and possibly better.

It was thought that possibly the introduction of an additional amount of sulfur to both absorption paths might be effective in desensitizing the instrument to sulfur. To test this possibility, a series of unleaded and leaded iso-octane standards was prepared

containing sulfur as thiophene. Unleaded iso-octane standards containing 0.002, 0.093, 0.473, 0.555, 0.913, and 0.979% sulfur were prepared and a series of leaded standards was prepared from each of these sulfur standards. The two series of leaded standards (2.0, 3.0, and 4.0 ml. of tetraethyllead per gallon) containing 0.002 and 0.093% sulfur were read against an equal volume (200 ml.) of unleaded iso-octane containing 0.002% sulfur in the reference beam. The two series of leaded standards (2.0, 3.0, and 4.0 ml. of tetraethyllead per gallon) containing 0.473 and 0.555% sulfur were read against an equal volume (200 ml.) of unleaded iso-octane containing 0.473% sulfur. Likewise, the two series of leaded standards (0.75, 2.5, 4.0, and 5.0 ml. of tetraethyllead per gallon) containing 0.913 and 0.979% sulfur were read against an equal volume (200 ml.) of unleaded iso-octane containing 0.913% sulfur.

The data obtained from the above schedule of absorbance measurements showed that the introduction of high sulfur levels (1.0% sulfur) in both beams does not desensitize the instrument to sulfur. This principle operates successfully for desensitizing infrared gas analyzers to certain components in a multicomponent mixture, because of the unique character and the lack of complete overlapping of the absorption bands of the various components in the infrared region of the spectrum. In the portion of the x-ray spectrum investigated (0.44 to 0.71 A.) the ratios of the mass absorption coefficients of lead and sulfur are nearly constant, as shown in Table XII. Thus, it does not seem probable that desensitization to sulfur can be accomplished in this manner.
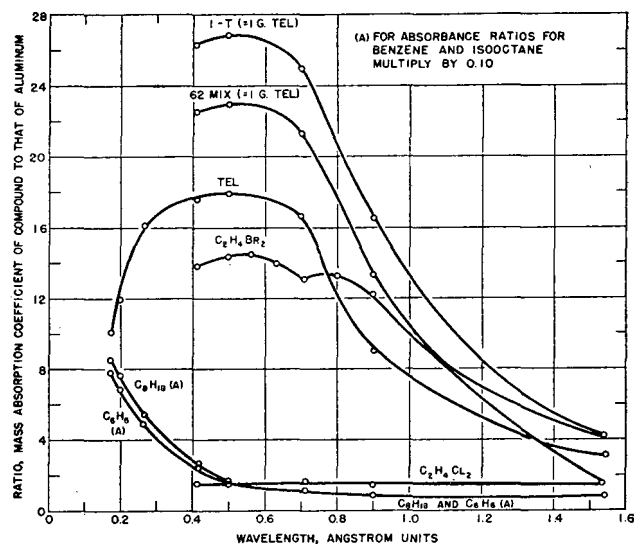

Figure 5. Relationships between Wave Length and Ratio of Mass Absorption Coefficients of Compounds to Aluminum

The data presented on the magnitude of the effect of sulfur and the variations in the absorbance of the base stocks by both methods (constant-weight and constant-volume) clearly show that these are the limiting factors of the absorption method. It was noted that for the most part there is a fair correlation of high and low tetraethyllead results with high and low sulfur content, in instances where sulfur analyses were available. This is obviously not the whole explanation of discrepant results, for the three lines shown in Figure 4 are not a true family of lines of constant sulfur content. Sulfur analyses are available on some of the base stocks represented: three samples of base stock A, 0.078, 0.063, 0.068%; two samples of base stock B, 0.028, 0.034%; base stock C, 0.034%; hot acid iso-octane, 0.002%; and a leaded iso-octane, 0.012% sulfur. Assuming that the middle line for benzene and iso-octane is representative of samples containing approximately 0.01% sulfur, then the upper

line is displaced at the proper distance for samples containing approximately 0.06 to 0.07% sulfur. It is difficult to explain the lower line, however, on the sulfur basis alone, because its displacement would require a "negative" sulfur content of 0.05 to 0.06%, compared with actual values of 0.035% sulfur. The location of the hot acid iso-octane sample in Figure 4 is also without explanation. Thus, it is obvious that while a correction based on sulfur content as proposed by Vollmar (12) would tend to give a more accurate tetraethyllead determination, there would still be erroneous results in unpredictable cases such as represented by the lower line of Figure 4.

CALCULATION OF CALIBRATION DATA FOR WAVE-LENGTH RANGE 0.417 TO 1.539 A.

A calculation was made of calibration data for tetraethyllead, as tetraethyllead, as 62 Mix, and as 1-T Mix in iso-octane over the wave-length range 0.417 to 1.539 A., from the mass absorption coefficient values (Table VIII) taken from the literature (9) in an effort to determine the optimum wave length for analysis and also to determine the practicability of differentiating the three kinds of tetraethyllead input experimentally by absorbance readings at more than one wave length. A calculation was also made of the quantitative effect of sulfur interference.

num were obtained. The ratios of the mass absorption coefficients are given in Table IX and those of the linear absorption coefficients in Table X.

The data in Table IX and X are also shown graphically in Figures 5 and 6, respectively. From Figure 5 it is noted that the ratios of the mass absorption coefficients of benzene to aluminum and iso-octane to aluminum are equal at a wave length of approximately 0.6 A.

Calculations were made of the linear absorption coefficients for iso-octane containing 1 ml. of tetraethyllead per gallon as tetraethyllead, as 1-T Mix, and as 62 Mix. The results obtained are given in Table XI. The calculated data of Table XI demonstrate the following points:

The values of percentage increase in linear absorption coefficients show that the greatest sensitivity for tetraethyllead determinations in a hydrocarbon base would be realized at a wave length of 0.710 A., the $K\alpha$ radiation of molybdenum. The method required would be one in which absorbance measurements were made directly in terms of current output from the receiver. These are the conditions used by Aborn and Brown (1); at the time of their work, however, the receiving system available was not adequate. This wave length is favorable as far as interference from sulfur is concerned (Table XII), and the μ_m of benzene and iso-octane are nearly the same at this wave length. Moreover, the use of monochromatic radiation would be a great advantage, because difficulties due to variations in effective wave length would be avoided.

The $\Delta\mu_l$ figures show that for an added increment of tetraethyllead, the increase in absorbance is appreciably greater at wave lengths of 0.710 A. and higher; the increase in absorbance of the hydrocarbon base, however, is relatively higher than that of tetraethyllead at wave lengths above 0.710 A. Thus, if the intensity of the radiation is sufficient to take care of the over-all increase in absorbance, the limit of detection of tetraethyllead would be greater at the higher wave lengths. Sullivan and Friedman (10), in using a molybdenum-target tube at 17 kv., were working at an effective wave length of approximately 0.94 A., close to the L_{III} absorption edge of lead. While the limit of detection is favorable at this wave length, the error introduced
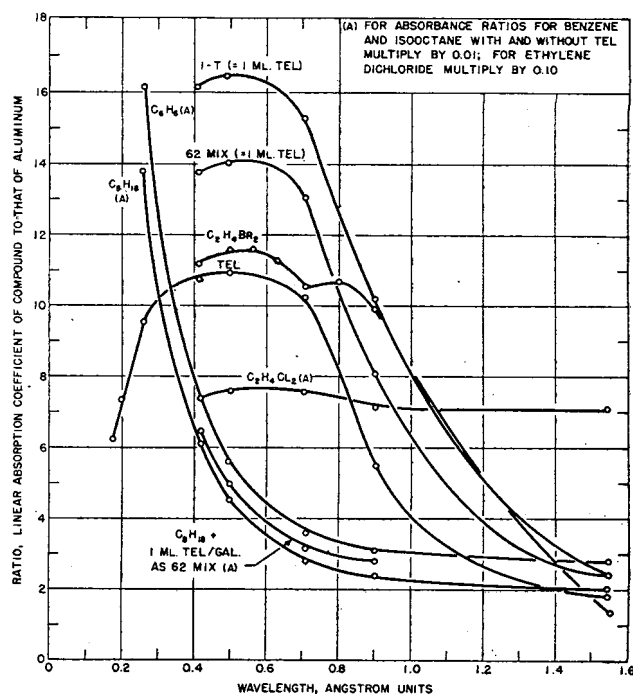
Table VIII. Mass Absorption Coefficients of Elements of Interest at Various Wave Lengths

λ, A.	C	H	Br[a]	Cl	Pb[b]	S	N	O	Al
					μ_m				
0.417	0.256	0.390	19.0	2.47	32.0	2.10	0.310	0.372	1.17
0.497	0.315	0.435	32.0	4.20	52.8	3.50	0.400	0.520	1.90
0.710	0.598	0.435	80.0	11.6	136.0	9.90	0.870	1.22	5.22
0.900	1.05	0.44	150.0	22.0[c]	145.0	19.3[c]	1.60[c]	2.35[c]	10.4
1.539	4.52	0.48	89.0	103.0	230.0	91.0	7.45	11.1	49.0

[a] Bromine has a K absorption edge at 0.918 A.; consequently, the coefficient is less at 1.539 than at 0.900 A.

[b] Lead has the following absorption edges: K at 0.138, L_I at 0.780, L_{II} at 0.813, and L_{III} at 0.950 A. These account for the closeness of the μ_m values at 0.710 and 0.900 A. For the x-ray absorption spectrum of lead see Sproull (9, p. 73).

[c] Values for chlorine, sulfur, nitrogen, and oxygen at 0.900 A. were obtained by interpolation from values given at wave lengths above and below 0.900 A.

Using the mass absorption data of Table VIII and the physical constants and conversion factors for the two standard commercial antiknock mixtures, the linear and mass absorption coefficients were calculated for the compounds and mixtures of interest in gasoline. Because all absorbance measurements in the present study were made in terms of aluminum, the ratios of the calculated linear and mass absorption coefficients to those of aluminum were obtained.

Table IX. Ratios of Mass Absorption Coefficients of Materials of Interest to Those of Aluminum

Ratio of μ_m^x to μ_m^{Al} Where x Is:

λ, A.	C₆H₆	C₈H₁₈	C₂H₄Br₂	C₂H₄Cl₂	TEL	1-T, eq. to 1 g. TEL	62 Mix, eq. to 1 g. TEL
0.417	0.227	0.237	13.85	1.58	17.59	26.37	22.48
0.497	0.171	0.176	14.35	1.64	17.85	26.93	22.90
0.710	0.112	0.110	13.05	1.62	16.71	24.99	21.37
0.900	0.096	0.092	12.28	1.55	8.96	16.55	13.22
1.539	0.086	0.079	1.56	1.53	3.03	4.05	4.02

Table X. Ratios of Linear Absorption Coefficients of Materials of Interest to Those of Aluminum

Ratio of μ_l^x to μ_l^{Al} Where x Is:

λ, A.	C₆H₆	C₈H₁₈	C₂H₄Br₂	C₂H₄Cl₂	TEL	1-T, eq. to 1 ml. TEL	62 Mix, eq. to 1 ml. TEL
0.417	0.074	0.061	11.18	0.734	10.77	16.17	13.79
0.497	0.056	0.045	11.59	0.760	10.92	16.48	14.02
0.710	0.036	0.028	10.54	0.754	10.23	15.30	13.08
0.900	0.031	0.024	9.92	0.717	5.48	10.13	8.09
1.539	0.028	0.020	1.26	0.710	1.86	2.48	2.46

Figure 6. Relationships between Wave Length and Ratios of Linear Absorption Coefficients of Compounds to Aluminum

Table XI. Linear Absorption Coefficients for Iso-octane Containing 1 Ml. of Tetraethyllead per Gallon

λ, A.	1 Ml. TEL/Gal. as TEL				1 Ml. TEL/Gal. as 1-T				1 Ml. TEL/Gal. as 62 Mix			
	μ_l	$\Delta\mu_l$	% inc.	$\Delta\mu_l/\mu_l^{Al}$	μ_l	$\Delta\mu_l$	% inc.	$\Delta\mu_l/\mu_l^{Al}$	μ_l	$\Delta\mu_l$	% inc.	$\Delta\mu_l/\mu_l^{Al}$
0.417	0.20193	0.00898	4.63	0.00284	0.20643	0.01348	6.96	0.00427	0.20445	0.01150	5.93	0.00364
0.497	0.24773	0.01480	6.35	0.00288	0.25526	0.02233	9.55	0.00435	0.25192	0.01899	8.12	0.00370
0.710	0.43700	0.03811	9.52	0.00270	0.45585	0.05696	14.25	0.00404	0.44760	0.04871	12.18	0.00346
0.900	0.70546	0.04064	6.08	0.00145	0.73992	0.07510	11.27	0.00267	0.72482	0.06000	9.00	0.00214
1.539	2.77410	0.06482	2.37	0.00049	2.79589	0.08661	3.17	0.00065	2.79519	0.08591	3.14	0.00065

Method used to calculate linear absorption coefficients for iso-octane containing 1 ml. of TEL per gallon for data at λ = 0.417 A.

Materials	C_8H_{18}	TEL	1-T Eq. to TEL	62 Mix, Eq. to TEL
μ_l	0.193	34.01	51.07	43.55
Volume %	99.9736	0.0264	0.0264	0.0264
$\frac{\text{Vol. \% } \times \mu_l}{100}$	0.19295	0.00898	0.01348	0.01150
μ_l of iso + 1 ml. TEL/gal.	...	0.20193	0.20643	0.20445
% increase in μ_l	...	4.63	6.96	5.93

by sulfur (Table XII) is almost twice as great as at wave lengths of 0.710 A. and lower.

The $\Delta\mu_l/\mu_l^{Al}$ figures are representative of the present operating conditions, since the General Electric instrument is used to measure the increase in absorbance due to tetraethyllead in terms of mils thickness of aluminum. These figures show that the optimum wave length is 0.497 A., and that there is very little variation in the range 0.417 to 0.710 A. Thus, considering the effect of sulfur interference, the relationship of μ_m of carbon to hydrogen, and the decrease in effective wave length due to filtration, the standardized conditions adopted for the present work are close to optimum for a double-beam instrument in which differences in absorbance are measured in terms of aluminum. The conditions are almost equally good at 0.710 A. Thus, filtered molybdenum $K\alpha$ radiation would be a very desirable substitute for the present polychromatic radiation from the tungsten target if the intensity of the radiation and/or the sensitivity of the receiving system were sufficient for satisfactory absorbance measurements. The data obtained with polychromatic radiation in this study clearly show the desirability of employing monochromatic radiation, if at all possible.

A comparison of the absorbance data for 1-T Mix and 62 Mix show that they are equal (within 1% of each other) at a wave length of 1.539 A. The reason for this is readily understood when one recalls that this is beyond the K absorption edge of bromine at 0.918 A. At first thought this appears to be one solution to the determination of tetraethyllead irrespective of whether it is present as 1-T Mix or 62 Mix. From Table XII, however, it is noted that the extremely high sulfur interference at this wave length makes the procedure impractical.

The calculations made of the equivalent absorbance of 0.10% sulfur in terms of milliliters of tetraethyllead per gallon as 62 Mix are given in Table XII.

Table XII. Linear Absorption Coefficients for Iso-octane Containing 0.10% Sulfur by Weight and Their Equivalence

λ, A.	$\mu_l^{C_8H_{18}} \times \frac{99.5}{100}$	$\mu_l^S \times \frac{0.05}{100}$	% Increase Due to 0.10% S	Eq., Ml. TEL/Gal. as 62 Mix
0.417	0.19290	0.00210	1.06	0.183
0.497	0.23288	0.00350	1.50	0.185
0.710	0.39880	0.00900	2.25	0.185
0.900	0.66467	0.01930	2.90	0.323
1.539	2.70865	0.09100	3.36	1.068

These calculated data confirm the experimental findings regarding the magnitude of the error due to varying sulfur content of gasolines. Experimentally it was found that the absorbance of 0.10% sulfur by weight is equivalent to 0.18 ml. of tetraethyllead per gallon as 62 Mix at an input voltage of 100, which corresponds to an effective wave length of approximately 0.50 A.

The ultimate goal is to measure the absorbance of lead without interference from bromine or sulfur. Theoretically this could be accomplished by making measurements with monochromatic radiation at wave lengths on both sides of the L_{III} absorption edge of lead at 0.950 A., the measurement on the short wave-

length side being at a wave length longer than the K absorption edge of bromine at 0.918 A. There appear to be two possible methods for doing this:

The use of filtered characteristic radiation from the proper target materials to give radiation at the two desired wave lengths. Theoretically, similar results might be obtained by the use of polychromatic radiation and balanced Ross filters such as discussed by Kirkpatrick (5) to give narrow pass bands at the two desired wave lengths.

The use of an x-ray spectrometer to obtain absorbance measurements on both sides of the L_{III} absorption edge of lead. Preliminary measurements made with the multiple-crystal spectrometer developed at Dow Chemical Company by L. K. Frevel and described by Liebhafsky (6) did not show a satisfactory resolution of the bromine absorption edge and the lead L_{III} absorption edge. Arrangements for similar tests with commercially available x-ray spectrometers have also been made.

Preliminary tests made with the General Electric XRD-3 spectrometer indicate that the bromine and lead absorption edges may be resolved and work is in progress to determine the sensitivity and accuracy which may be attained.

The limiting factors to these two approaches to the problem may be a lack of intensity of radiation, sensitivity of the receivers, and stability and reproducibility of electronic balancing circuits.

APPARENT ANOMALIES IN ABSORBANCE MEASUREMENTS

During the course of this work there have been several instances of absorbance measurements which were difficult to explain, because they appeared to be contradictory to the known mass absorption coefficient data and the laws of absorption. It was later recognized, however, that the apparent anomalies were the result of two effects—namely, change in effective wave length due to filtration, and loss of intensity due to scattering. While the two effects are interdependent and therefore difficult to study individually, it has been possible to make some measurements which magnify one effect in preference to the other.

Variations in Effective Wave Length with Quantity and Quality of Absorbers. When polychromatic radiation passes through an absorbing medium, the longer wave lengths are preferentially absorbed, because the absorption coefficients of the elements increase with the third power of the wave length and the fourth power of the atomic number except at the positions of the sharp absorption edges. For this reason the effective wave length of the emergent radiation as well as the traversing radiation varies with the quantity and quality of the absorbing elements. This effect has been well discussed by Liebhafsky (7) in relation to similar studies and by Clark (2) under the general heading of filtration as it applies to industrial and medical radiography.

The method described by Liebhafsky (7) has been used to determine the effective wave length of the polychromatic radiation before and after passing through various absorbers. With the present instrument this was done by blocking one beam with lead and measuring the peak intensity of the other beam under specified conditions, both with and without a small increment of aluminum. In making the peak intensity measurements, the amplification meter was used as an intensity meter. It is then possible to calculate the experimental mass absorption coefficient of aluminum (7, Equation 3), which may then be converted to the corresponding effective wave length from known mass absorption coefficient vs. wave-length data. Using this procedure the effective wave lengths of the initial radiation at an input voltage of 100, and that of the radiation after traversing various absorbers have been determined. Results are given in Table XIII.

While the data of Table XIII were not obtained under optimum conditions for measuring effective wave length, they are considered to be sufficiently accurate to give a reliable comparison of the filtration caused by the various absorbers. These data show that for an increased tetraethyllead content of 6.0 ml. of tetraethyllead per gallon of iso-octane there is an appreciable decrease in the effective wave length. However, as the wave length decreases from 0.440 to 0.400 A. (Table IX and Figures 5 and 6) the mass absorption coefficients for benzene and iso-octane relative to aluminum are increasing, while those for tetraethyllead are decreasing. These effects evidently compensate one another very closely, since straight-line relationships were obtained for tetraethyllead concentration vs. absorbance. This analysis of the resulting effective wave lengths with increasing tetraethyllead concentration serves to show that the straight lines obtained are in reality a fortunate coincidence. Moreover, under conditions where the ratio of tetraethyllead to hydrocarbon is greatly increased, the resulting relationship could very well be a curved line. Such a relationship has been obtained on the standardization of a method for the determination of total lead, where the ratio of lead to hydrocarbon is many times higher than it is in commercial gasoline.

Table XIII. Effective Wave Length after Traversing Various Absorbers

(Input voltage 100)

Description of Sample	Sample Beam	$\lambda_{eff.}$, A.
Blank	R.H.	0.500
50-mil Al block	R.H.	0.460
50-mil Al block	L.H.	0.458
150 g. iso-octane	R.H.	0.440
150 g. benzene	R.H.	0.432
Polystyrene block (5.7 cm.)	R.H.	0.454
Polystyrene block (5.7 cm.) + 50-mil Al block	R.H.	0.429
Polystyrene block (5.7 cm.) + 50-mil Al as attenuator	L.H.	0.427
Polyethylene block (2.7 cm.)	R.H.	0.468
200 ml. iso-octane	R.H.	0.440
200 ml. iso-octane + 3.0 ml. TEL/gal.	R.H.	0.429
200 ml. iso-octane + 6.0 ml. TEL/gal.	R.H.	0.403
125 mils of aluminum	R.H.	0.432

An interesting comparison to be made from Table XIII is that of the 125 mils of aluminum and the polystyrene block, which has an absorbance equivalent to 125 mils of aluminum. The aluminum decreases the effective wave length more than does the hydrocarbon. Actually the measured absorbance of the hydrocarbon is partially due to scattering and not entirely to true absorption. Thus, while the measured absorbance is the same as the aluminum it does not decrease the effective wave length of the transmitted radiation to the same extent.

It was disturbing to find that with the introduction of the polystyrene block in the reference beam, the sensitivity values in Table III were reduced to about 80% of the values obtained when the absorbances of the samples were balanced entirely by aluminum in the reference beam. Likewise, the ratio of the absorbance in mils of aluminum to the density of the hydrocarbons was reduced to 80% of the value obtained when aluminum was the sole absorber in the reference beam. The data presented in Table XIV were obtained to illustrate this point and also to show that the same condition holds if the hydrocarbon absorber in the reference beam is iso-octane. It is apparent from Table XIV that when the reference beam contains a hydrocarbon absorber as well as some aluminum, the absorbance is less in terms of mils of aluminum to balance the same absorber in the right-hand beam. As shown previously, the shorter the effective wave length, the greater the relative absorbance of hydrocarbon to aluminum, and the more aluminum in the reference beam (the aluminum attenuator and the aluminum blocks, when needed, precede the polystyrene block

in the optical path), the shorter the effective wave length before it enters the polystyrene absorber. Because the effective wave length is decreased at a faster rate by aluminum than by hydrocarbons (Table XIII), these factors acting together result in the decreased absorbance readings in terms of mils of aluminum noted in Table XIV.

An experiment was performed to show the magnitude of the effect produced on absorbance measurements by progressively decreasing the effective wave length. Absorbance measurements were obtained on 50-gram samples of benzene and iso-octane both before and after the addition of successive 10-gram increments. The results are shown graphically in Figure 7. The amount of absorbance for each successive increment increased up to a total weight of 160 grams for iso-octane and 170 to 180 grams for benzene, after which there was little change. With increasing amounts of the hydrocarbons the effective wave length decreases and the absorbance relative to aluminum increases (Figure 6). Furthermore, Table XIII shows that for hydrocarbon and aluminum of equivalent absorbance (5.7-cm. polystyrene block and 125 mils of aluminum) the effective wave length is decreased more by aluminum than by hydrocarbon. Thus, as more and more aluminum is added to balance the absorbance of the added increments of hydrocarbons, the effective wave length of the radiation passing through the aluminum absorber is decreasing at a rate faster than that of the radiation passing through the hydrocarbon absorbers. Therefore, the increase in mils of aluminum required to balance the successive increments of hydrocarbons is readily understandable. Unless some other factor is operating, this trend would be expected to continue, whereas actually a definite plateau is noted. This phenomenon is due to geometric considerations and a scattering effect, as discussed in the next section.

Loss of Intensity Due to Scattering. Absorbance measurements have been obtained which demonstrate a loss of intensity of radiation due to scattering by hydrocarbons. Furthermore, the data show the effect of varying the position of the absorber in the x-ray beam on the loss of intensity. In this study a series of measurements was made on the 2.7-cm. polyethylene block when placed in the absorption cell at successively higher levels. The data obtained are given in Table XV and shown graphically in Figure 8. It is apparent from curve D that the shorter the distance from the hydrocarbon block to the receiver (fluorescent screen), the lower the measured absorbance, and from the curve marked $1/D^2$ it is clear that the decrease in measured absorbance is inversely proportional to the square of this distance.
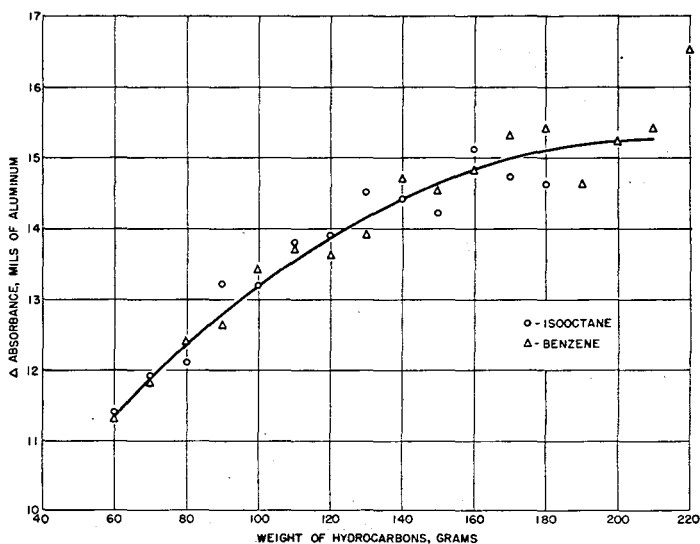

Figure 7. Absorbance of Added Increments of Hydrocarbon

Table XIV. Comparison of Results Obtained by Using Various Absorbers in Reference Beam

| Absorber in Reference Beam | Absorbance, Mils of Aluminum | | Δ |
	200 ml. iso-octane	200 ml. iso + 3.0 ml. TEL/gal. as 1-T[a]	
Aluminum	162.4	227.1	64.7
Al + polystyrene block (5.7 cm.)	27.1 (eq. to 152.1)[b]	79.6 (eq. to 204.6)[b]	52.5
Al + 150 ml. iso-octane	36.5	88.9	52.4

[a] Polystyrene block has absorbance equivalent to 125.0 mils of aluminum.
[b] Iso-octane plus 3.0 ml. TEL/gal. as 1-T used was not freshly prepared standard and therefore has low absorbance value.

Table XV. Absorbance of Polyethylene Block at Different Positions in X-Ray Beam

Distance from Bottom of Block to Fluorescent Screen, Cm.	$1/D^2$	Absorbance, Mils of Aluminum
25.25	0.0015	44.0
20.75	0.0023	43.8
17.75	0.0032	43.5
14.75	0.0046	43.2
11.75	0.0072	42.4
8.75	0.0131	41.4

Because the measured absorbance is the sum of true absorption and scattering (2, 3, 11) it has been theorized that the farther the absorber is from the receiver (the smaller the subtended solid angle) the greater the loss in intensity due to scattering and consequently the greater the measured absorbance. The linear relationship (Figure 8) obtained experimentally between the loss of measured absorbance (decrease in loss of intensity due to scattering) and the reciprocal of the square of the distance from the absorber to the receiver is a gratifying confirmation of this theory.

The experiment was repeated with the 48-mil aluminum block. The absorbance readings were the same, however, regardless of the distance of the block from the receiver. This constancy of readings indicates there is no measurable scattering of radiation by aluminum. It was therefore decided to calculate the per cent of the absorption due to scattering for aluminum and for carbon at the various wave lengths of interest. Details of these calculations and tables of contents used for expediting them are given by Victoreen (11). The calculated values of total mass absorption coefficients (μ_m) and mass scattering coefficients (σ_m) are given in Table XVI. From these data it is seen that the per cent of absorption due to scattering by carbon at 0.50 A. is 54.6, while that by aluminum is only 9.1. Thus these calculations explain the effect of scattering noted with the hydrocarbon block and its absence with aluminum. The effect was further verified by the relative darkening of photographic films positioned so as to register the scattered radiation from the polyethylene and aluminum blocks.

Table XVI. Per Cent Absorption Due to Scattering

| λ, A. | μ_m | | σ_m | | $\sigma_m/\mu_m \times 100$ | |
	Carbon	Aluminum	Carbon	Aluminum	Carbon	Aluminum
0.5	0.336	1.950	0.1835	0.1769	54.6	9.1
0.6	0.449	3.226	0.1870	0.1803	41.6	5.6
0.8	0.812	7.318	0.1910	0.1841	23.5	2.5
1.0	1.402	13.95	0.1920	0.1851	13.7	1.3
1.2	2.280	23.68	0.1948	0.1870	8.5	0.8

Because loss of intensity due to scattering is less the closer the absorber is to the receiver, it is postulated that this effect is working in opposition to the increase of absorbance with decreasing effective wave length under the conditions noted previously in Figure 7. As the level of the hydrocarbon increases in the cell with each additional 10-gram increment, the scattering effect decreases and thus the measured absorbance decreases.

This dependence of absorbance on the position of the hydrocarbon absorber in the x-ray beam also accounts for the lower

absorbance value for iso-octane than for benzene when 150-gram aliquots were used (Table V). From Figure 5 it will be noted that the ratios of the mass absorption coefficient of iso-octane to aluminum are actually greater than those for benzene to aluminum at wave lengths below 0.63 A. However, because the densities of benzene and iso-octane are 0.883 and 0.701, respectively, the level of the iso-octane would be 25% higher than that for benzene in the absorption cell for equal-weight aliquots. Thus it is realized that for a 25% increase in height of the hydrocarbon absorber, there is an appreciable decrease in the measured absorbance due to a decrease in the scattering effect at the higher levels in the absorbance cell. This, therefore, demonstrates that the scattering effect could contribute some error in absorbance measurements if the constant-weight method were to be applied to gasolines having densities significantly higher or lower than average.
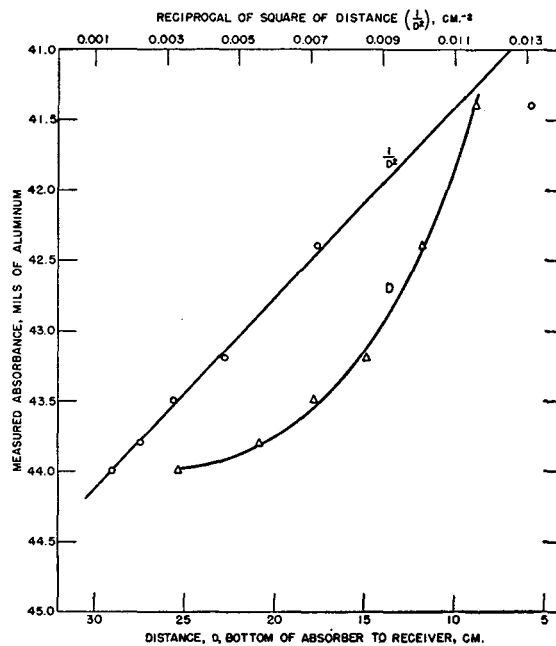

Figure 8. Effect of Position of Polyethylene Block in X-Ray Beam on Loss of Intensity Due to Scattering

Thus it is seen that the various apparent anomalies encountered in absorbance measurements may be attributed to a filtering and/or a scattering effect.

CONCLUSIONS

The results obtained in this investigation show that x-ray absorptiometry is suitable for the rapid determination of tetraethyllead in gasoline with the following limits of accuracy:

Known base stocks of known sulfur content or base stocks available for blank determination: ±0.01 ml. of tetraethyllead per gallon.
Unknown base stocks of known sulfur content: ±0.03 ml. of tetraethyllead per gallon.
Aviation gasoline (unknown saturated base stock of low sulfur content): ±0.03 ml. of tetraethyllead per gallon.
Unknown base stocks of a presumed sulfur content of 0.03 to 0.15%: ±0.11 ml. of tetraethyllead per gallon (based on calibration data for base stocks containing 0.09% sulfur).

These limits of accuracy are based on the assumption that known, constant lead-to-halogen ratios are maintained.

Tetraethyllead determinations made on 68 gasoline samples by the constant-volume method showed an over-all precision of ±0.01 ml. of tetraethyllead per gallon, and on comparison with chemical analysis an average error of ±0.05 ml. of tetraethyllead per gallon. Data obtained by the constant-weight method indicate

that equal accuracy and precision may also be obtained with this method.

All data obtained in this study indicate that variation in the sulfur content of gasoline is the major limiting factor on the accuracy of tetraethyllead determinations by x-ray absorptiometry when using polychromatic radiation. It was found both experimentally and by calculation that a sulfur content of 0.10% results in tetraethyllead determinations which are high by 0.24, 0.18, or 0.16 ml. per gallon when the tetraethyllead is present as tetraethyllead, 62 Mix, or 1-T Mix, respectively.

It was concluded that in order to make an independent correction for sulfur and other possible base stock variables, it would be necessary to make absorbance measurements with monochromatic radiation on both sides of an absorbance edge of lead. It was further pointed out that if the L_{III} absorbance edge of lead at 0.950 A. were selected and if the measurement on the low wavelength side were between 0.918 and 0.950 A., it would be possible to obtain lead determinations free from halogen as well as free from sulfur interference.

ACKNOWLEDGMENT

The authors wish to thank the following members of the staff of the Ethyl Corporation Research Laboratories: J. D. McCullough for his work in making instrument modifications which resulted in marked improvement in sensitivity, reproducibility,

and stability; Gordon W. Wilcox and Leonard M. Niebylski for the preparation of the standards of tetraethyllead in hydrocarbons.

LITERATURE CITED

(1) Aborn, R. H., and Brown, R. H., IND. ENG. CHEM., ANAL. ED., 1, 26 (1929).
(2) Clark, G. L., "Applied X-Rays," 3rd ed., New York, McGraw-Hill Book Co., 1940.
(3) Compton, A. H., and Allison, S. K., "X-Rays in Theory and Experiment," 2nd ed., New York, D. Van Nostrand Co., 1949.
(4) Gross, W., and Staab, F., Deutsche Luftfahrtforschung, Forschungsbericht Nr. 1476, Air Documents Div., T-2, AMC, Wright Field Microfilm No. R2744, F1248.
(5) Kirkpatrick, P., Rev. Sci. Instruments, 10, 186 (1939).
(6) Liebhafsky, H. A., ANAL. CHEM., 21, 17 (1949).
(7) Liebhafsky, H. A., Smith, H. M., Tanis, H. E., and Winslow, E. H., Ibid., 19, 861, 866 (1947).
(8) Rich, T. A., and Michel, T. C., Gen. Elec. Rev., 50, No. 2, 45 (February 1947).
(9) Sproull, W. T., "X-Rays in Practice," New York, McGraw-Hill Book Co., 1946.
(10) Sullivan, M. V., and Friedman, H., IND. ENG. CHEM., ANAL. ED., 18, 304 (1946).
(11) Victoreen, J. A., Applied Phys., 20, 1141 (1949).
(12) Vollmar, R. C., Petterson, E. E., and Petruzzelli, P. A., ANAL. CHEM., 21, 1491 (1949).
(13) Zemany, P. D., Winslow, E. H., Poellmitz, G. S., and Liebhafsky, H. A., Ibid., 21, 493 (1949).

RECEIVED May 11, 1950.

Tetraethyllead in Gasoline

X-Ray Absorption Spectrometry for Determination of Tetraethyllead in Gasoline

Investigation of Mono- and Polychromatic Methods and Description of Monochromatic Method for Routine Use

HAROLD K. HUGHES, Technical Service Department, Socony-Vacuum Laboratories, Brooklyn 22, N. Y., AND FRANK P. HOCHGESANG, Research and Development Department, Socony-Vacuum Laboratories, Paulsboro, N. J.

Various x-ray absorption methods have been investigated experimentally with respect to their sensitivity for the determination of tetraethyllead, their freedom from interference by sulfur and base gasoline variations, and their practical usefulness. A monochromatic method using two thorium x-ray lines near a lead absorption discontinuity is recommended as a reasonably foolproof, rapid method for routine use. In the absence of commercially available thorium-target x-ray tubes, a monochromatic method employing one molybdenum x-ray line is currently in use. A sample is analyzed in less than 7 minutes with an accuracy that averages better than 0.1 ml. of tetraethyllead per gallon. When tetraethyllead is blended into a gasoline of known stock, the maximum error should equal the reproducibility, which is 0.03 ml. of tetraethyllead per gallon. This method is compared to chemical and polarographic methods with respect to speed, accuracy, and cost.

PETROLEUM companies in the United States devote so many thousands of man-hours each year to tetraethyllead determinations that there is need for a more rapid and economical method than the chemical one currently standardized by the American Society for Testing Materials (3, D 526–48T). Polarographic methods are contributing to the solution of this problem and have been reported (7, 13).

The purpose of this paper is to describe an investigation of x-ray absorption methods applicable to this problem. One of these is now in routine use and proving to be adequately accurate for the analysis of commercial gasolines. It is extremely rapid, a single determination requiring only 7 minutes.

Chemical analyses and determinations by x-rays are not particularly new; von Hevesy (14) published a book on the subject 18 years ago. It is only recently, however, that further developments in this field have been stimulated by the availability of suitable commercial equipment and by the increasing interest in more rapid and economical methods of analysis. Recently, Engström (12) gave a good survey of both the emission and absorption possibilities of x-ray analysis. Friedman (5) and co-workers have investigated x-ray fluorescent spectra for analytical use.

As far as the authors are aware, Aborn and Brown (1) performed the first x-ray determination of tetraethyllead in gasoline

in 1929, using the total radiation from a molybdenum target tube and an ionization detector. Their procedure was improved in 1946 by Sullivan and Friedman (21), who substituted a Geiger counter for the ionization chamber and reduced the anode voltage to 17 kv. to increase the sensitivity. More recently, the General Electric Company has put on the market an x-ray spectrophotometer (8, 11, 16, 18, 25–27). It operates on white radiation from tungsten and attempts to reduce the large effects of voltage fluctuations by the use of a split beam. Voltage fluctuations change the effective wave length (27) and so change the mass absorption coefficients, as shown by Figures 1 and 2.

Others (4, 17, 19) have also proposed x-ray absorption for chemical determinations. The Dow automatic x-ray absorption spectrometer is mentioned briefly in Liebhafsky's review (15).
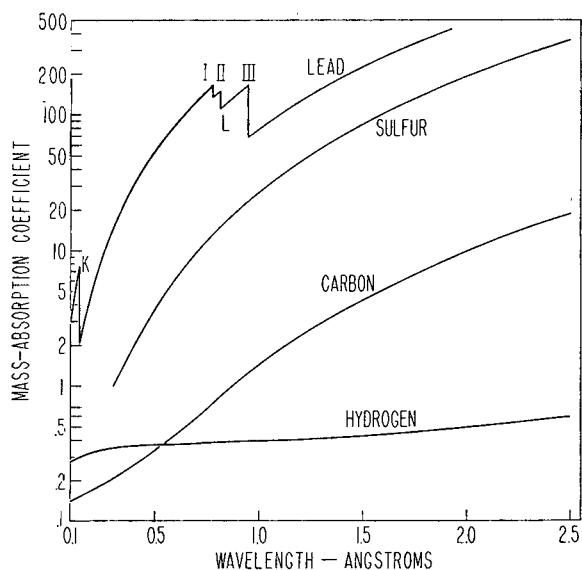
Figure 1. Variation of Mass Absorption Coefficient with Wave Length for Lead, Sulfur, Carbon, and Hydrogen (2, 10, 24)

From the standpoint of practical methods of analysis, x-ray absorption procedures may start with either a continuous or a line source of radiation. The choice between these is, in many respects, similar to the choice between a colorimeter and a spectrophotometer, the important considerations being sensitivity, specificity, reliability, and cost.

In the choice of a suitable wave length for maximum sensitivity, reference should be made to Figures 3 and 4, in which are plotted the ratios of the mass absorption coefficients of lead, bromine, and chlorine to the mass absorption coefficient of carbon. Tetraethyllead fluid contains all three of the former elements and, for maximum sensitivity, it is desirable that the absorption which they produce be as large a fraction of the total as is possible. Obviously, carbon always accounts for the greatest part of the attenuation of the beam by gasoline.

Interferences enter into x-ray and spectrophotometric methods similarly and their complete elimination is impossible with either continuous or single-line methods. Of course, two-component analyses can be done with two wave lengths. Sulfur is the most important interfering element.

INVESTIGATION OF MONO- AND POLYCHROMATIC METHODS

In the investigation of continuous-radiation and monochromatic methods, results were sought in terms of sensitivity for the determination of tetraethyllead in gasoline and interference of sulfur, because the latter is considered to be the most variable unknown present, especially in experimental automotive gaso-

lines. The total radiation was from tungsten and molybdenum target tubes, while molybdenum and thorium tubes supplied the monochromatic radiation. The potential was about 36 kvp. in each case. The possibility of using a tetramethyllead-filled Geiger counter, as a detector with increased sensitivity toward lead, was also investigated.

INSTRUMENTATION

The tungsten and molybdenum continuous radiations were obtained from air-cooled, hot-filament x-ray tubes supplied with Lindemann glass windows. These were operated on a Norelco x-ray spectrometer, as supplied, except that the line voltage to the unit was stabilized by a 1-kv.-amp. Sola constant-voltage transformer. This system contains no rectification except that inherent in the x-ray tube and operates with about 36 kvp. across the x-ray tube. The anode current averages 5.6 ma.

Thorium line radiation was obtained from a cold cathode x-ray tube with aluminum foil windows constructed according to the design of Chesley (9). Potential was supplied to this tube from a half-wave rectifier built in this laboratory. The tube was operated at 36 kvp., anode water-cooled and grounded, with the tube current varying with pressure about a 2.8 ma. average. A thorium pellet, 0.5 cm. in diameter, was pressed and soldered into the copper cooling head. Thorium rod from which the pellet was machined was supplied by A. D. Mackay Chemicals, New York, N. Y. Adequate provision must be made to prevent ingestion of thorium.

Difficulty was experienced in obtaining good heat conductivity within the thorium and/or from thorium to copper. Apparently, this was due to the presence of thorium in a sintered form which contained some oxide. Vacuum heat treatment of the thorium pellet would be expected considerably to improve heat conductivity and perhaps the ductility, but facilities to accomplish this were not available. Selected thorium pellets did provide satisfactory operation for periods of a few days each, after which the target and the cold cathode (aluminum) had to be removed and resurfaced to remove pits.
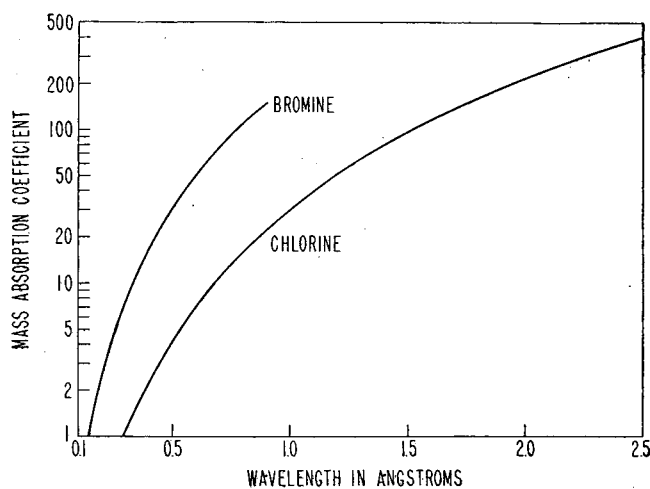

Figure 2. Variation of Mass Absorption Coefficient with Wave Length for Bromine and Chlorine (2, 10, 24)

For the total radiation experiments the beam emerging from the x-ray tube was stopped down with a pinhole aperture placed on the Norelco spectrometer housing at the exit port (approximately 4 cm. from the focal spot). The diaphragms were made by drilling holes in 1/8-inch (3.18-mm.) sheet lead, 0.020 inch (0.508 mm.) in diameter for molybdenum and 0.0625 inch (1.59 mm.) for tungsten. A sliding shutter was located at the exit side of the pinhole. The Geiger counter was aligned in the divergent beam emerging from the pinhole near the x-ray tube with the counter entrance window about 12 inches (30 cm.) from the focal spot. The beam was further stopped down with a pinhole of 1/8-inch sheet lead placed in front of the Geiger counter entrance window; this was 0.0313 inch (0.795 mm.) in

diameter for molybdenum and 0.125 inch (3.18 mm.) for tungsten. Cells made from brass pipe approximately 1 inch (2.54 cm.) in inside diameter were then located centrally in the beam defined by the pinholes. Windows for the cells were cleaved from sheet mica, selected for comparable transmittance and mechanically clamped to the ends of the brass-pipe cells. Cells were filled through a hole in the pipe. Cleaning after emptying a sample was accomplished by washing several times with petroleum ether and air-drying before filling with a new sample. Cells were checked frequently to ensure comparable transmittance between the blank and sample cells.
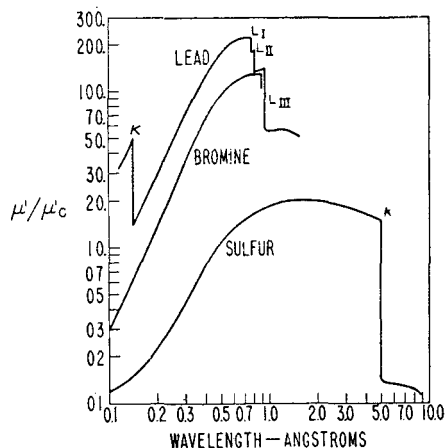
Figure 3. Variation of Ratio of Mass Absorption Coefficient of Lead, Bromine, and Sulfur to Carbon

In these measurements, the reference radiation level, P_0, was always measured through a blank cell filled with gasoline (or n-heptane where appropriate). The P_0 measurement was thus corrected for absorption by the hydrocarbon components. Especially in the total radiation experiments where cells 20 cm. long were used, the quality of the radiation "filtered" by this length of hydrocarbons is considerably different from that emerging from the x-ray tube.

Monochromatic radiation was obtained using a single crystal of rock salt, slightly roughened on a cleaved face. Absorption cells were placed between the crystal and the Geiger counter housing. The detectors used were argon- and tetramethyllead-filled Geiger counters. The argon counter with mica window was supplied as a standard item by the North American Philips Company and was found to have its plateau near 1400 volts. The tetramethyllead-filled counter was constructed of glass and had a blown "bubble" window. Its absorbing path was roughly 20 cm. long and it was filled with tetramethyllead to a pressure of 2.5 cm. of mercury, after which 61 cm. of mercury pressure of helium was added. The plateau for this counter was found near 1900 volts. The x-ray beam was stopped down until the counting rate through the blank cell filled with gasoline or n-heptane was within the range of 75 to 200 counts per second.

X-ray beam intensities encountered under the conditions described above are sufficient to overload the counters badly unless the x-ray beam is suitably decreased in intensity. Conditions of operation must be checked to ensure that the linear response region of the counters is being used. Finally, adequate precautions to prevent body exposure to either direct or scattered x-ray radiation must be observed.

RESULTS AND DISCUSSION

Both total tungsten and total molybdenum radiations were ound to provide adequate sensitivity for the determination of tetraethyllead in gasoline, as had been reported by others (*1, 8, 16, 18, 21, 25-27*). Results, as shown in Figures 5 and 6 and ummarized in Table I, are reported to enable direct comparison with the monochromatic methods. Total molybdenum radiation was investigated to decide whether the intense K-lines near 0.7 A. would yield greater sensitivity for lead compared to the essen-

tially continuous radiation from tungsten. Slightly increased lead sensitivity was observed but this gain was offset by increased sulfur interference; 0.10 weight % sulfur in gasoline was found to be equivalent to 0.14 and 0.17 ml. of tetraethyllead per gallon for total tungsten and total molybdenum radiations, respectively. Others (*8*) have reported, using the General Electric x-ray photometer with total tungsten radiation at comparable voltage, that 0.10 weight % sulfur is equivalent to 0.19 ml. of tetraethyllead per gallon. This difference may be due to the detectors employed and is not considered significant. The argon counter used in these experiments probably had less sensitivity for long wave-length radiation relative to short wave-length radiation than the phosphor-phototube detector employed in the G.E. instrument, but this point has not been investigated. Inherent filtration by the x-ray tube windows and cell windows, plus scattering of the x-ray beam, is not to be neglected, but these conditions are believed to be reasonably similar for the two experiments mentioned above.

Figure 5 also illustrates the fact that detector sensitivity does cause marked differences in the slope of the analytical curves. The tetramethyllead-filled Geiger counter, rather than exhibiting an increased slope for lead as was hoped, actually was found to have a lesser slope. This is undoubtedly because the glass window of the tetramethyllead counter absorbed the longer wave-length radiation in greater amount than the mica window of the argon counter.
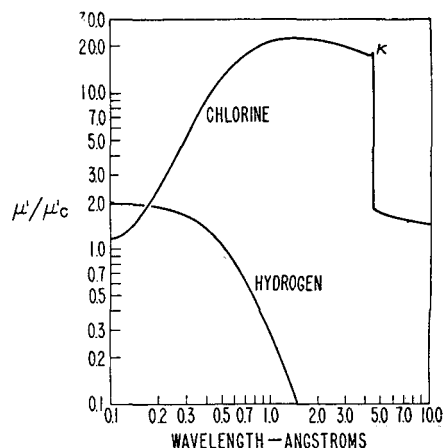

Figure 4. Variation of Ratio of Mass Absorption Coefficient of Chlorine and Hydrogen to Carbon

Line radiation monochromatized by a single reflection from a cleaved rock salt crystal is believed to offer an important advantage over total radiation methods, in that the quality (spectral distribution) of the radiation remains stable for minor variations of voltage across the x-ray tube whereas the quality of the continuous spectrum changes. The molybdenum $K\alpha$ and $K\beta$ lines (0.71 and 0.63 A., respectively) were found to provide increased absorbance for lead, so that shorter cells, and thus lesser volumes of sample, were required (see Figure 7 and Table I). However, sulfur interference is essentially the same as for the total radiation experiments reported above. For the molybdenum line radiations 0.10 weight % sulfur was found to be equivalent to 0.15 ml. of tetraethyllead per gallon.

The molybdenum $K\alpha$ line, available from diffraction equipment on hand in many laboratories, does offer a convenient basis for a rapid, practical method for the determination of tetraethyllead in gasoline. Such a method has been developed and is presented in detail below.

Obviously, the desired freedom from sulfur interference can be obtained by making measurements across an absorption dis-

continuity for lead, the L-edge near 0.85 A. being convenient for this purpose. However, instruments to record continuous absorption spectra are not generally available. In order to make measurements in a short time with equipment of moderate cost, a line spectral source which provided lines near an absorption "peak" and an absorption "trough" for lead was investigated.
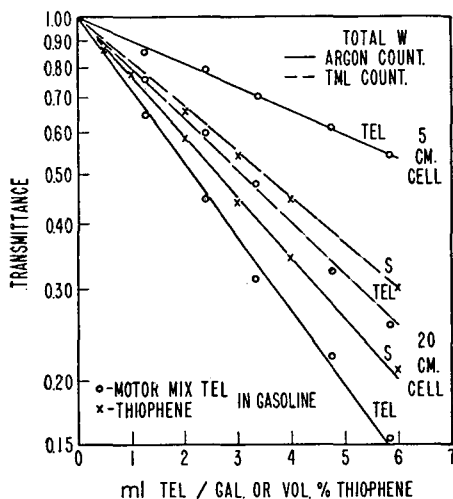

Figure 5. Transmittance vs. Concentration of Tetraethyllead and Thiophene in Gasoline

Total tungsten radiation, argon- and TML-filled Geiger counters, 5- and 20-cm. cell thicknesses

A thorium x-ray tube operated at 36 kvp. provides lines of adequate intensity at 0.76 and 0.96 A. Results obtained are shown in Figures 8 and 9. These lines are separated in wave length much too far to assume that the absorption due to the sulfur is constant. However, because the sulfur-tetraethyllead mass absorption coefficient ratio does change nearly fourfold (Table I), both sulfur and lead can be determined simultaneously, assuming only that variations in the base gasolines introduce no other errors. At 0.96 A. sulfur absorbs strongly, whereas lead has become relatively more transmitting. From the transmittance, τ, of a given gasoline measured at these two wave lengths

both the sulfur and tetraethyllead concentrations can be calculated by solution of two-component simultaneous equations as follows:

$$-\log \tau_{0.76} = Ac_T + Bc_S \tag{1}$$

$$-\log \tau_{0.96} = Dc_T + Ec_S \tag{2}$$

where c_T = concentration of tetraethyllead in milliliters per gallon, c_S = concentration of sulfur in weight per cent, and A, B, D, and E = constants determined from known samples. For motor mix tetraethyllead fluid in n-heptane the following values were found experimentally:

$A = 0.0909$ $D = 0.0408$
$B = 0.148$ $E = 0.250$
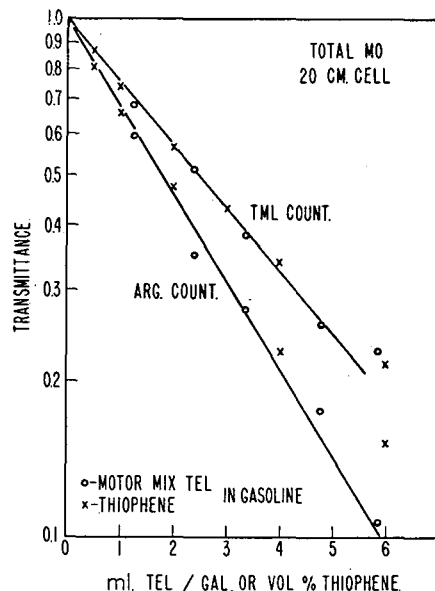
Figure 6. Transmittance vs. Concentration of Tetraethyllead and Thiophene in Gasoline

Total molybdenum radiation, argon- and TML-filled Geiger counters, 20-cm. cell thickness. Points for both motor mix TEL and thiophene fall near same straight line

The gas-type, thorium target, x-ray tube used for these initial experiments is unstable as compared to the evacuated, hot-filament tubes normally used for diffraction studies and thus is not recommended as an x-ray source for routine analytical measurements. It is probable that a hot-filament tube could be constructed and would offer greater stability. To illustrate the possibilities of the method, a sample known to contain 1.25 ml. of tetraethyllead per gallon and 0.089 weight % sulfur (0.15 volume % thiophene) was run four times with the following results:

Run	$\tau_{0.76}$	$\tau_{0.96}$	c_T, Ml. TEL/Gal.	c_S, Wt. % S
1	0.747	0.842	1.24	0.095
2	0.751	0.827	1.13	0.142
3	0.749	0.845	1.23	0.089
4	0.745	0.843	1.26	0.089

For the above measurements, as well as for all calibrating runs, the thorium tube was operated at 12 to 13 microns of mercury pressure (Pirani-type gage) which allowed a tube current of 2.8 ma. at 36 kvp. The counting rates observed for P_0 (through 5-cm. cells filled with n-heptane) were about 90 and 165 counts per second for the 0.96 and 0.76 A. lines, respectively. In order to obtain measurements of the desired accuracy 3-minute counts were made for each sample and blank. Blanks and samples

Table I. Summary of Experimental Results

Radiation[a]	Detector	Cell Length, Cm.	Ml. TEL/Gal. Equivalent to 0.10% Wt. S[b]	Absorptivity[c] Motor mix	Sulfur	Ratio S/TEL
Total W	Argon	20[d]	0.14	0.00708	0.0100	1.41
Total W	TML	20[e]	0.15			
(Total W)	Phosphor phototube	15[f]	(0.19)			
Total Mo	Argon	20[d]	0.17	0.00826	0.0144	1.74
Total Mo	TML	20[e]	0.17			
Mo 0.63 A.	Argon	9.6[d]	0.151	0.0120	0.0181	1.51
Mo 0.71 A.	Argon	9.6[d]	0.148	0.0162	0.0241	1.49
Th 0.76 A.	Argon	5[g]	0.162	0.0182	0.0293	1.61
Th 0.96 A.	Argon	5[g]	0.618	0.00807	0.0498	6.16

[a] All radiation generated at approximately 36 kvp.
[b] This factor depends upon density—for example, 1 wt. % S = 1.74 vol. % thiophene in gasoline used = 1.68 vol. % thiophene in n-heptane.
[c] Absorptivity, a, is defined by equation $P/P_0 = 10^{-abc}$, where b is cell length in cm. and c is concentration (for motor mix expressed as ml. of TEL/gal. and for sulfur as wt. %).
[d] Lindemann glass window x-ray tube; mica window cells; mica window, argon-filled Geiger counter.
[e] Same as [d] except tetramethyllead counter had window blown from Corning 9741 glass.
[f] Aluminum window cells, phosphor-phototube detector. Ethyl Corp. results (8).
[g] Same as [d] except aluminum window x-ray tube.

were measured alternately and the blank counts before and after each sample were averaged to establish P_0.

The molybdenum 0.63 and 0.71 A. lines, which are more readily available, unfortunately cannot be used to determine both lead and sulfur simultaneously because the sulfur-tetraethyllead mass absorption coefficient ratio is essentially the same at these two wave lengths (see Table I).
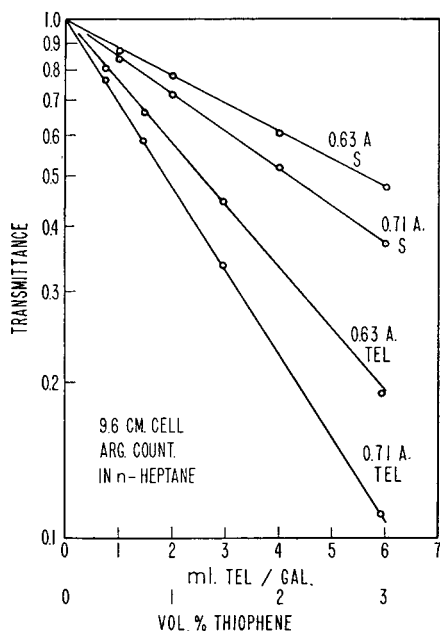

Figure 7.   Transmittance vs. Concentration of Tetraethyllead and Thiophene in n-Heptane

Molybdenum monochromatic radiation ($K\alpha$ and $K\beta$ lines), argon counter, 9.6-cm. cell thickness.   Thiophene scale is expanded compared to Figures 5 and 6

From the data obtained in the above survey, mass absorption coefficients for sulfur and lead were calculated. The values found are summarized in Table II. The calculated values for both sulfur and lead are smaller than the values reported in the literature, the deviation increasing with wave length. Some evidence was found to indicate that at a given wave length the deviation increases with cell length, but these data are incomplete at present.

The absorption contributions by lead, bromine, and chlorine in motor mix tetraethyllead have not been separated in these experiments. In one method of computation, it is assumed that the hydrocarbon portion of the motor mix fluid is similar to a paraffinic hydrocarbon and, especially at the low concentrations involved, that the only difference between P_0 measured through n-heptane or blank gasoline and P measured through the sample (containing motor mix fluid at 3 ml. of tetraethyllead per gallon) is due to lead, bromine, and chlorine. In order to calculate the mass absorption coefficient for lead from these experimental data the contributions by bromine and chlorine are assumed to conform to the literature values. This method of calculation requires that any deviation from the true value be accounted for in the calculated μ' for lead, whereas bromine and chlorine values probably deviate also.

A preferred method of stating the deviation for tetraethyllead fluid from ideal conditions is to assume literature values of the mass absorption coefficients for lead, bromine, and chlorine and then to calculate the cell length. These values are reported in the last column of Table II. They are found to deviate in the

same manner as the calculated absorption coefficients but to a lesser extent, as was expected.

Possible explanations for the observed deviation include lack of spectral purity of radiation "monochromatized" by a single crystal reflection, scattering with no change of wave length, and scattering with a change of wave length—i.e., fluorescence. The extent to which these possible mechanisms contribute to the deviation has not been investigated in detail. The most intense source of spectral impurity would be expected to be second-order radiation reflected by the crystal. The deviation increases with wave length and is in the correct direction to be thus explained. However, even at 0.63 A. some deviation is noted, whereas no $\lambda/2$ radiation (0.32 A.) should be present from an x-ray tube operated at 36 kvp. ($\lambda_{min.} = 12,400/36,000 = 0.34$ A.). The operating voltage was measured with an electrostatic voltmeter and found to be 26 kv. root mean square voltage which, provided the wave form was sinusoidal, would equal 36 kvp. Transients may be present to supply a higher peak voltage and thus some $\lambda/2$ radiation, even for the 0.63 A. measurements. Certainly second-order radiation is present in the measurements made at longer wave lengths, but the extent to which this is important is undetermined.

Concerning energy dissipated by scattering, Sproul (20) comments that scattering is a minor factor in the absorption of x-rays by heavy elements but becomes important for the lighter elements. For example, in lead, scattering dissipates more energy than is truly absorbed only when the generating potential exceeds 500 kv. But in carbon, scattering dissipates more energy than that which is truly absorbed when the tube voltage exceeds about 25 kv. This indicates that carbon would be expected to be the "worst" scattering offender in this case. But because P_0 was always measured through a blank gasoline (or n-heptane) in exactly the same experimental arrangement as when a sample P was measured, it would appear that scattering due to carbon was essentially the same for the two measurements.
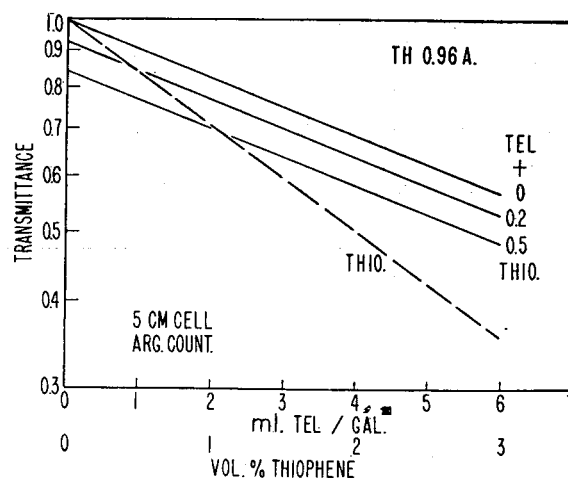

Figure 8.   Transmittance vs. Concentration of Tetraethyllead and Thiophene in n-Heptane

0.96 A. thorium monochromatic radiation, argon counter, 5-cm. cell thickness

Whatever the source of the observed deviations in mass absorption coefficients may be, the effect presents no limitation for analytical use of the method except to indicate that all working curves should be established with standard samples and interferences checked experimentally. Calculations based upon true mass absorption coefficients serve only to indicate the approximate sensitivity for the desired and interfering elements. In practical analytical use the deviation decreases the sensitivity

Table II. Comparison of Mass Absorption Coefficients with Literature Values

Radia-tion, A.	Cell Length, Cm.	Sulfur Calcd.	Sulfur Lit.[a]	Sulfur Δ, %	Lead Calcd.[b]	Lead Lit.[a]	Lead Δ, %	Calcd. vs. Known Cell Length, %[c]
0.63	9.6	6.31	6.90	−9	76	98	−22	−18
0.71[d]	10.5	8.45	9.90	−15	114	136	−16	−16
0.71	9.6	8.45	9.90	−15	101	136	−26	−20
0.76	5	9.84	12.2	−19	102	170	−40	−30
0.96	5	16.9	22.9	−26	[e]	65		[e]

[a] Literature values from, or interpolated from, Compton and Allison (10), calculated values from $P/P_0 = \exp(-\mu'\rho b)$ where μ' is mass absorption coefficient, ρ is density in grams per ml., and b is path length in cm.
[b] μ' for lead, calculated assuming that bromine and chlorine contributions to absorption by motor mix TEL fluid conform to literature values for μ' for these elements.
[c] Deviation of calculated cell length for sample containing 3 ml. TEL/gal., no sulfur, from actual cell length. Calculated length assuming that lead, bromine, and chlorine contributions to absorption by motor mix TEL fluid conform to literature values for μ' for these elements.
[d] Calculated from one-line molybdenum method.
[e] Impossible to calculate $\mu'Pb$ or cell length at 0.96 A. owing to lack of μ' for bromine at this wave length.

slightly, thereby requiring a longer absorbing path length than that calculated from true mass absorption coefficients.

As shown by Figure 14, a careful check of the effect of sulfur upon the absorption reveals that this deviation from elementary theory, whatever its explanation may be, enters into the basic absorption equation only as a factor, less than one, multiplying the cell length. This factor is a constant for any fixed cell length and wave length; it is independent of concentration. Thus, a plot of the logarithm of the transmittance versus the concentration is still a straight line.

SUMMARY AND CONCLUSIONS

X-ray absorption analysis approaches that "push-button" analytical goal wherein a property is measured rapidly on a sample in the condition as received and with practically no opportunity for errors of human judgment. No chemical manipulation of the sample is required. Moreover, several analyses of interest to the petroleum industry can be made on equipment currently available for x-ray diffraction use with only minor modification. However, the gain in speed and freedom from manipulation may be at the expense of specificity.

The applications of x-ray absorption to analysis and control can be grouped into several broad classes:

As a method specific for the determination of a given element in samples of only generally similar over-all composition—for example, the determination of tetraethyllead and sulfur in gasoline wherein sulfur is the major unknown variant. Such specificity, essentially independent of base gasoline composition, can be achieved only by measuring the transmittance at two wave lengths, such as across an absorption discontinuity for the element concerned. Because the "workable" region of the x-ray spectrum is at present severely limited (approximately 0.5 to 2.5 A.) only a few elements may be considered. In the region from roughly 0.5 to 2.5 A., K-discontinuities occur for elements from atomic numbers 22 through 47, titanium through silver; L-discontinuities occur for atomic number 52, tellurium, and heavier elements. The shortest wave-length discontinuity (K) for lighter elements—e.g., sulfur 16, phosphorus 15—occurs at a wave length longer than 2.5 A. at which most cell or x-ray tube windows absorb strongly, air scatters appreciably, and the radiated intensity is low. The determination described herein, of tetraethyllead and sulfur in gasolines, of unknown base stock using thorium 0.76 and 0.96 A. radiation, is a specific example of this type of rapid, yet reasonably foolproof method.

As a method specific for the determination of a given element in samples wherein it is the only variant and the over-all composition is known or the "blank" is available for direct comparison. This unique situation, which is met in blending operations (25), is admirably suited to precise, instantaneous, rugged, and relatively inexpensive control by x-ray absorption. The blending of tetraethyllead into gasoline or metal-organo additives into fuels and lubricants should be a natural field for control by this means.

As a method nonspecific for the determination of a given element in samples wherein it is the major variant and the over-

all composition varies only within known limits. The rapid, routine determination of tetraethyllead in current production gasolines by total x-ray radiation or by the one-line molybdenum method described in detail in the following section is an example of this general field of application. The "one-line moly" method offers advantages of better spectral purity and consequently increased accuracy and reproducibility of analysis compared to total radiation methods such as the x-ray photometer employing total tungsten radiation. An analogy might be drawn between such x-ray methods and the use of a mercury lamp as compared to white light for the determination of the absorption of a blue dye. Caution must be observed in applying x-ray absorption methods to this type of nonspecific determination, since the presence of unknown metallic impurities can cause considerable interference without any clue that interference is present. However, the reproducibility, rapidity, and low cost of x-ray absorption for this latter field of application suggest its use whenever possible.

MONOCHROMATIC METHOD FOR ROUTINE USE

The procedure employing the $K\alpha$ radiation from a molybdenum target tube, which was mentioned briefly above, has been in routine use for several months. Operating cost is low compared to methods currently in use; thus this method has been investigated extensively with respect to details of manipulation and accuracy.

APPARATUS

The apparatus used is a Norelco x-ray spectrometer (North American Philips Company, Mount Vernon, N. Y.) modified as shown in Figure 10. The absorption cell is placed between the crystal monochromator, which is in the position ordinarily occupied by the diffraction sample, and the Geiger counter detector housing.
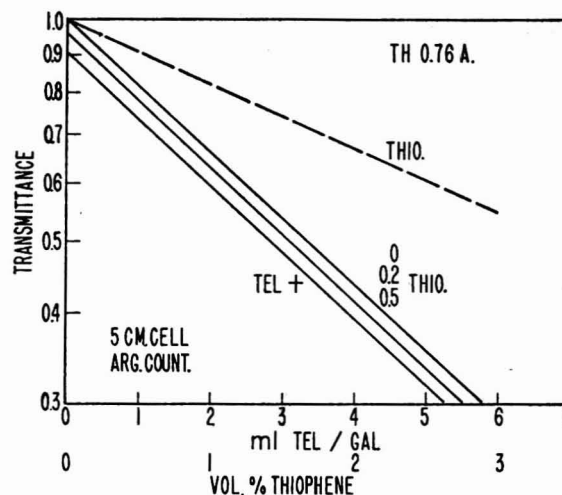
Figure 9. Transmittance vs. Concentration of Tetraethyllead and Thiophene in n-Heptane

0.76 A. thorium monochromatic radiation, argon counter, 5-cm. cell thickness

Power is supplied through a Sorensen voltage regulator (Sorensen & Company, Inc., 375 Fairfield Ave., Stamford, Conn., Model 1750, 2 kv.-amp.). This regulator should be loaded to near its rated capacity in order to minimize the effect of transients created by half-wave operation of the x-ray tube. The molybdenum target x-ray tube is air-cooled and operated at 35 kvp., 5.4 ma., without supplementary rectification. Radiation leaving the mica window of the x-ray tube is partially collimated by a $^1/_{32}$-inch (0.80-mm.) diameter pinhole aperture placed in the exit slit housing. This beam is monochromatized to obtain the $K\alpha$ radiation ($\lambda = 0.71$ A.) by diffraction from the "400" plane of a rock salt crystal. This target, of those which are readily available commercially, gives the best ratio of lead to carbon absorption as shown in Figure 3. The L_I absorption edge of lead occurs at 0.781 A.

For ease of adjustment to obtain maximum intensity the crystal is held in a goniometer head (Charles Supper Company, 28 Union St., Newton Center 59, Mass.) as shown in Figure 10. This has two

calibrated screw-adjusted arcs for angular motion and two dove-tail screw adjustments for the translational movements. Two additional adjustments—namely, rotation about and translation along a vertical axis—are provided by the brass assembly supporting the goniometer head. The latter was designed at these laboratories. This assembly consists of a brass bed plate, containing a recess, in which the goniometer head, screwed to the graduated disk, moves freely. A shaft located below the bed plate fits snugly in the hole provided in the spectrometer. A steel circular collar, fastened to the shaft by means of setscrews, controls the vertical height of the crystal goniometer. Completing this assembly is a long dural rod 7.5 inches (19 cm.) in length, attached to the bed plate by a setscrew. The other end, not seen in Figure 10, is spring-loaded against a 10-32 screw which effects a very fine rotational adjustment of the crystal. A disk attached to this fine screw is graduated in 12 parts; one twelfth of a revolution of the screw causes approximately a 1-minute circular displacement of the crystal. This has been found to be sufficiently sensitive.
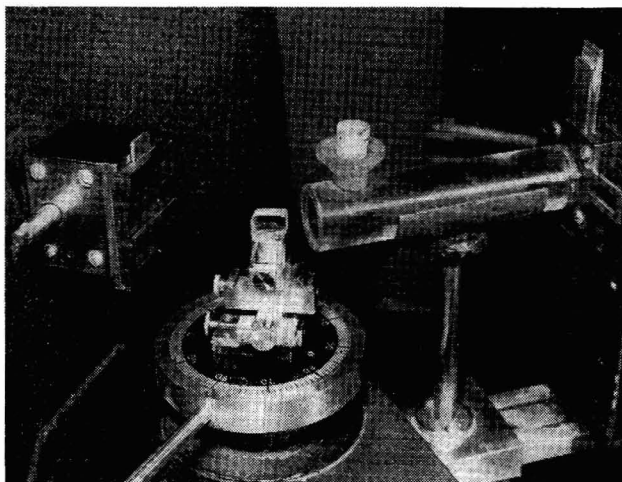
Figure 10. Apparatus for X-Ray Absorption Measurements

Left to right. **Exit slit on x-ray tube housing, cleaved rock salt crystal on goniometer, x-ray absorption cell in supporting cradle, entrance slit on Geiger counter housing**

Pentaerythritol, quartz, calcite, graphite, beryllium, and rock salt were investigated as crystal monochromators. (Pentaerythritol crystals may be purchased from the General Electric X-Ray Corporation, Milwaukee 14, Wis.; quartz and calcite from Ward's Natural Science Establishment, Rochester, N. Y.; graphite from the National Carbon Company, Niagara Falls, N. Y.; beryllium from the Machlett Company, Springdale, Conn.) Rock salt was chosen for this work because of its ruggedness, crystalline simplicity (face-centered cube), and the strong intensity of its reflections. The reflection from the "400" plane at $2\theta = 29.26°$ is used as the monochromatic beam in the absorption methods for tetraethyllead with $\lambda = 0.71$ A. The crystal is spray-coated with clear plastic to reduce the effect of water vapor.

A picture and drawing of transparent (XMP-60) plastic and opaque dural absorption cells, 8 ml. in volume, may be seen in Figures 11 and 12, respectively. At each of the cells are beryllium windows, approximately 0.017 inch (0.43 mm.) thick, which are attached by means of cement. The transmittance of a pair of beryllium windows to molybdenum K_α radiation is about 95%. Preference is given to the transparent cell, because any trapped air may be seen. The cell is stoppered during the course of the analysis to minimize evaporation which has been found to cause significant changes in absorption. An angular cut from the bottom of the funnel to one end of the cell minimizes the possibility of accumulating air bubbles.

The absorption cell holder (Figure 10) consists of a brass support of fixed height fastened at the base to the Geiger counter arm by a knurled screw. A lead cradle, shaped to the contours of the absorption cell, is attached to the platform. The center of the window of the cell coincides with the center of the slit and crystal assembly. The radiation is detected by a chlorine-quenched, argon-filled counter (Philips Type 62019). This has a high transmittance mica window, a threshold of about 1425 volts, and an operating potential of approximately 1475 volts.

Located at the entrance to the Geiger counter is a slit assembly containing horizontal and vertical metal slides. Adjustment to the beam height is accomplished by a *horizontal slide* bearing a wedge-shaped aperture graduated in seven steps of 1 mm. each, from 0 to 7 mm. Adjustment to the beam width is effected by a vertical slide containing three fixed apertures which are 0.011 inch (0.28 mm.), 0.020 inch (0.51 mm.), and 0.040 inch (1.02 mm.) wide. These slit assemblies are seen in Figure 10.

Quanta are counted by a decimal scaler preset for a fixed count of 10,000 (Model 1000, Berkeley Scientific Company, Sixth & Nevin Aves., Richmond, Calif.). The electronic scale-of-1000 gives direct decimal indication of all counts, without interpolation. A four-place built-in register records thousands. An automatic scaling unit is plugged into the accessories socket on the Berkeley decimal scaler and provides automatic cut-off for a preset number of counts (Berkeley extended-range automatic scaling unit, Model 820). The scaler may be operated for any multiple of 1000 counts up to 39,000 and for any multiple of 100 counts up to 3900. When the preset number of pulses has been registered, the scale automatically stops counting and timing. A self-starting electric clock, having a range of 6000 seconds, readable to 0.1 second, is plugged into the extended range automatic scaling unit. (Type S 100, manufactured by the Standard Electric Time Company, Springfield, Mass., has been found satisfactory.) The clock starts when the button on the scaling unit is depressed; this also starts the counting by the decimal scaler.

Numbers inversely proportional to the initial radiant power (P_0) are obtained by placing a standard absorber of nickel, 0.005 inch (0.13 mm.) thick, in the slot provided in the Geiger slit assembly. No shifting of the "400" reflection from sodium chloride is noted when using nickel foil for this purpose.

OUTLINE OF PROCEDURE

The crystal of rock salt is sealed with Duco cement to the top of the crystal goniometer as shown in Figure 10. It is then centered with respect to the beam along the vertical and longitudinal axes and the Geiger counter positioned at $2\theta = 29.26°$. Following these coarse settings the crystal goniometer is adjusted for maximum intensity with respect to rotation about the vertical axis, translation across the beam, and rocking about the axis perpendicular to the beam. Of these three, the first is by far the most critical but is easily accomplished by means of the 10-32 spring-loaded screw previously described. A rocking motion of the crystal about an axis parallel to the beam is also available, but this is not very critical and movement of the pinhole exit slit vertically is somewhat more convenient. In the final setting, the Geiger counter is also moved slightly from its computed setting, which takes into account the effective thickness of the crystal and the alignment error of the spectrometer goniometer. After some practice, this alignment can be completed in 5 minutes.

Timing for a fixed number of counts, 10,000 in this case, is used to measure the radiant power of the transmitted and "zero" beams. This has the merit of constant statistical error for all readings. The choice of 10,000 counts is a compromise between conserving time for an analysis and reducing the error due to statistical fluctuations. It takes somewhat more than 60 seconds for 10,000 counts of Mo K_α radiation to be transmitted

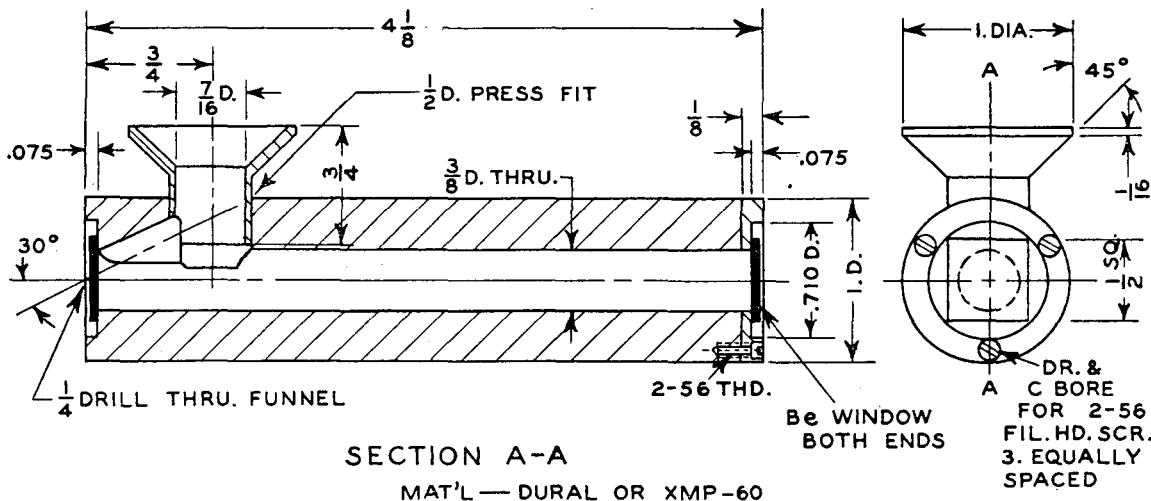**Figure 11. Opaque and Transparent Absorption Cells**

Figure 12. Details of Construction of Absorption Cell
Dimensions in inches

through a 10-cm. absorption cell containing iso-octane. It is believed that "gagging" of the counter on Mo $K\alpha$ radiation starts to be appreciable in the neighborhood of 10,000 counts per minute.

As shown in Equations 5 and 6, the density, ρ, must be known in order to determine the concentration of tetraethyllead in gasoline. In most cases, the density measurements are performed routinely before the samples are submitted for tetraethyllead analysis. Thus, it is important that the temperature be known at the time of their analysis as appropriate corrections must be made. The magnitude of the density error is presented in Table VII.

DETAILS OF METHOD

1. Fill and stopper the cell carefully with the gasoline sample (approximately 8 ml.) in such a manner as to avoid trapping air.
2. Determine the temperature of the sample to the nearest degree while step 3 is in progress.
3. Place the standard nickel absorber in the appropriate slot before the Geiger tube. Open beam shutter. Depress button on the extended range automatic scaling unit, preset to 10,000 counts, to start the recording of counts as well as the timer. Determine t_0, the time for 10,000 counts. Close beam shutter and remove standard absorber.
4. Place the cell on the sample holder so that one of the ends rests against the Geiger slit assembly. Open beam shutter and determine the time, t, as in step 3. Close beam shutter and remove cell.
5. Repeat step 3 to obtain another value for t_0. The average t_0 is computed from steps 3 and 5.
6. While step 5 is in progress, wash the absorption cell three times with small portions of the next sample to be analyzed and then fill with it.
7. Repeat steps 4 and 5. Determine the average t_0 from the values found in steps 5 and 7. Thus it is seen that for a series of n samples, $n + 1$ determinations of t_0 are made in order to obtain each average t_0.
8. The value of t/t_0, obtained from the analysis of the sample, locates a point on the calibration curve (Figure 13). This yields an abscissa value from which the tetraethyllead concentration of the sample may be determined. A nomograph is employed to reduce this computation to a minimum.

Separate curves are needed for automotive and aviation gasolines because of different amounts of halogens present in the mixes.

PREPARATION OF CALIBRATION CURVE

The radiant power, P, of a narrow pencil of parallel and monochromatic x-rays decreases exponentially as it passes through a sample of thickness b, density ρ, and mass absorption coefficient μ', according to the general equation

$$\tau = \frac{P}{P_0} = e^{-\mu'\rho b} \tag{3}$$

where τ is the fractional transmittance. The exponent in this equation is termed the absorbance. When the sample is a mixture of materials, μ' is calculated as

$$\mu' = \sum_i \mu'_i f_i \tag{4}$$

where f_i is the weight fraction of component i in the mixture. The compositions of tetraethyllead mixtures are given in Table III.

For purposes of computing the effective mass absorption coefficients of automotive and aviation mixes, it is necessary to know the weight percentages of the five principal elements present in these mixtures. These have been computed from Table III and are given in Table IV.

Employing the mass absorption coefficients depicted in Figures 1 and 2, and listed in Table V for the molybdenum $K\alpha$ radiation which is employed in this method, Equation 3 becomes

$$(P/P_0) \text{ automotive gas} = e^{-b[\rho(0.587 + 9.31 f_s) + 0.0478 c_T]} \tag{5}$$

$$(P/P_0) \text{ aviation gas} = e^{-b[\rho(0.587 + 9.31 f_s) + 0.0555 c_T]} \tag{6}$$

in which c_T is the lead concentration in units of milliliters of tetraethyllead per gallon of finished gasoline.

Table III. Compositions of Tetraethyllead Mixtures
Per cent by weight, 8)

	Automotive Mixture	Aviation Mixture
Tetraethyllead	61.48	61.41
$C_2H_5Br_2$	17.86	35.68
$C_2H_2Cl_2$	18.81	None
Kerosene	1.7[a]	2.8[a]
Dye	0.124[a]	0.0809[a]

[a] Approximate.

Table IV. Elemental Composition of Tetraethyllead Mixtures
(Per cent by weight)

	Automotive Mixture	Aviation Mixture
Hydrogen	4.8	4.7
Carbon	26.7	25.3
Chlorine	13.8	None
Bromine	15.4	30.7
Lead	39.4	39.3

Equations 5 and 6 show that a plot of log (P/P_0) versus either $\rho(0.587 + 9.31fs) + 0.0478c_T$ or $\rho(0.587 + 9.31fs) + 0.0555c_T$, depending upon the type of gasoline, is a straight line.
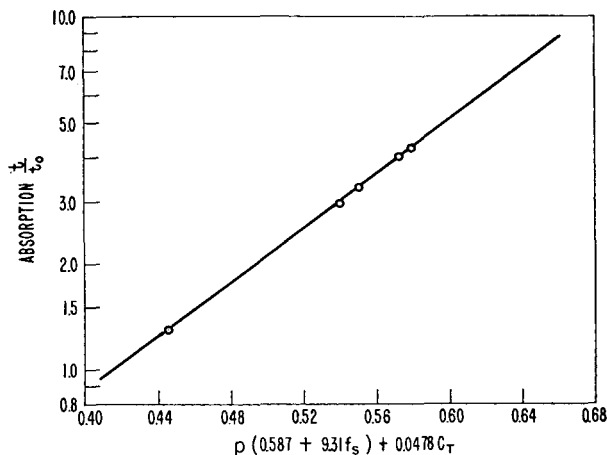

Figure 13. Working Curve for Determination of Tetraethyllead in Automotive Gasolines

t, time for fixed count of 10,000
ρ, density
fs, weight fraction of sulfur
cT, concentration of TEL, ml./gallon

Five automotive gasoline samples of known sulfur and tetraethyllead concentrations are analyzed as explained above. The average t/t_0 values so determined are plotted as ordinates on semilog paper. The abscissa values are calculated as $\rho(0.587 + 9.31fs) + 0.0478c_T$. Figure 13 shows a typical calibration curve. The tetraethyllead values used were obtained by the polarographic method. Sulfur values were determined by the A.S.T.M. method D 90–47T. A similar plot for tetraethyllead in aviation gasolines, not shown here, may be constructed by plotting t/t_0 against $\rho(0.587 + 9.31fs) + 0.0555c_T$ on semilog paper.

As a test of the above general theory particularly with respect to the effect of sulfur upon the transmittance of light products to x-rays, a series of experiments was run in which sulfur in various forms was blended at known concentration levels into four different solvents. These solvents were chosen from the four classes: paraffins, olefins, aromatics, and naphthenes. The five series which were run and which are plotted in Figure 14 are as follows: thiophene in "iso-octane" (2,2,4-trimethyl-pentane), dibutyl sulfide in iso-octane, dimethyl disulfide in 1-heptene, dibutyl sulfide in benzene, and dibutyl sulfide in cyclohexane. From the known composition of the sulfur compounds and the μ' of carbon, hydrogen, and sulfur, μ'_p of the compounds was computed as follows: thiophene 4.15, dibutyl sulfide 2.62, and dimethyl disulfide 6.93. The abscissa values in Figure 14 are computed from the equation

$$\mu'_{blend} = \frac{0.622r + 0.381}{1 + r} + \left(\mu'_p - \frac{0.622r + 0.381}{1 + r} \right) \frac{fs}{fs_p} \quad (7)$$

where r = carbon to hydrogen ratio, fs_p = weight fraction of

sulfur in the compound, and fs = weight fraction of sulfur in the blend.

$$\rho\mu'_{blend} = \rho(P + Q fs) \quad (8)$$

It is seen from Figure 14 that the experimental results do yield essentially a straight-line relationship, thereby indicating that the effects of sulfur and carbon-to-hydrogen ratio upon this one-line method are as predicted by their elemental absorption coefficients. Incidentally, Figure 14 shows that an analysis for sulfur may be made when the carbon-to-hydrogen ratio is known (or approximated) and when metals, halogens, etc., are absent.

CORRECTION FOR SULFUR CONTENT

Automotive and aviation gasoline stocks contain small amounts of sulfur compounds which absorb x-rays strongly and which are read as additional tetraethyllead if allowance is not made for their presence. When the sulfur content is known, as in blending operations, or a separate sulfur determination is run for other purposes, the exact correction is made. In other classes of samples, however, the concentration of sulfur may be unknown and resort is made to an average correction based upon a statistical analysis of samples drawn from the same classifications. These average values are adjusted from time to time as new factors enter the picture. It is known that the sulfur content also varies with geographical area, so that each laboratory needs to determine its own correction.

The average sulfur concentration shown in the most recent data published by the Bureau of Mines (6) is 0.064% for 152 samples collected in Districts 1, 2, 3, and 4 comprising Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Delaware, Maryland, Virginia, North Carolina, South Carolina, Georgia, Alabama, Florida, Tennessee, Ohio, West Virginia, and Eastern Kentucky. These data are tabulated in Table VI and presented in Figure 15. They are now one year old and it is believed that the average value today is somewhat higher. Corresponding data for aviation gasolines are not available but are known to be generally lower.

OTHER POSSIBLE ERRORS

Of the remaining errors which may occur in x-ray tetraethyllead determinations, change in the sample due to evaporation is by far the most serious. This is not peculiar to the x-ray method, but its extreme importance has been recognized only recently because of the almost "instantaneous" nature of the test.
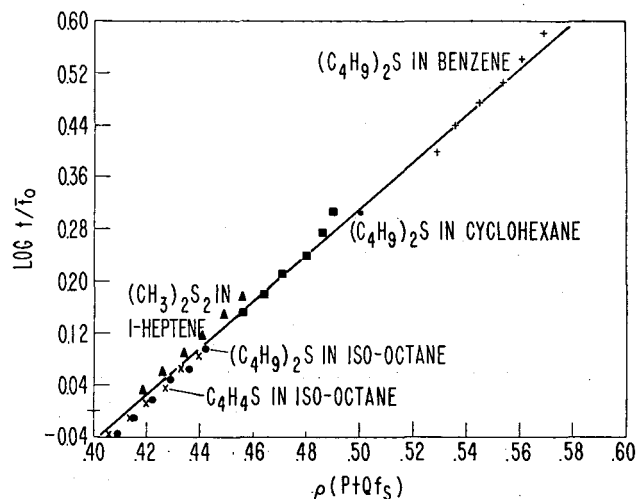
Table V. Mass Absorption Coefficients (2, 22–24)

	MoKα, λ = 0.710 A
1 hydrogen	0.381
6 carbon	0.622
16 sulfur	9.90
17 chlorine	11.6
35 bromine	80
82 lead	136

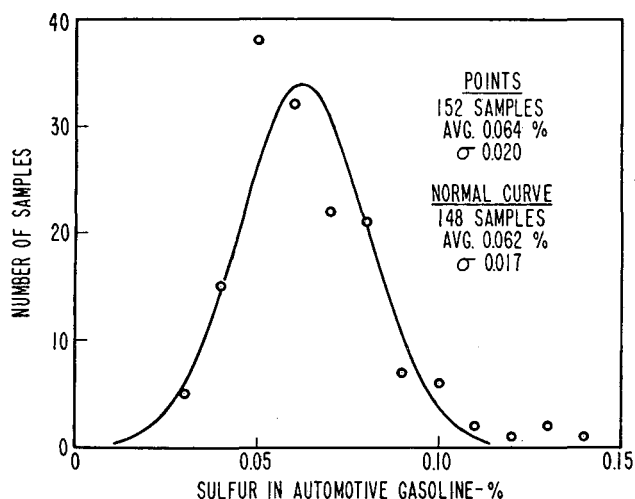**Figure 14. Effect of Sulfur in Hydrocarbons upon Transmittance**

Figure 15. Statistical Plot of Sulfur Content of Automotive Gasolines in Eastern United States, Winter 1948–49 (6)

Indeed, the course of evaporation can be followed easily with a counting-rate meter. With automotive gasolines, the change is always in the direction of increasing tetraethyllead concentration and may amount to over 35% in extreme cases. Prevention of evaporation is thus extremely important, especially with winter gasolines of high vapor pressure.

ACCURACY AND SPEED OF ANALYSES

One would not expect the over-all error to be a simple summation of those listed in Table VII, but to average around some value moderately larger than the greatest one. It is reasonable to predict that the average error will be less than 0.1 ml. per gallon in most cases. When the base stock and sulfur content are known or constant, this error can be reduced to about 0.03 ml. of tetraethyllead per gallon. The error in the polarographic method is believed to be about 0.05 ml. per gallon, while the ultimate standard method (A.S.T.M. D 526–48T) has a listed accuracy (3) of between 0.04 and 0.06 ml. per gallon, depending upon the concentration. Other possible errors and the magnitude of their contributions are listed in Table VII.

Table VI. Grouped Values for Sulfur in Gasoline, Eastern United States, Winter 1948–49, Bureau of Mines Survey (6)

% Sulfur	No. of Samples
0.03	5
0.04	15
0.05	38
0.06	32
0.07	22
0.08	21
0.09	7
0.10	6
0.11	2
0.12	1
0.13	2
0.14	1
	152

Average sulfur concentration, 0.0642%
Standard deviation, σ, 0.020%

To develop empirical information on both the accuracy and the speed of the methods, a large number of laboratory samples, chosen at random, have been analyzed. Table VIII presents the results of one recent series of 25 tetraethyllead determinations. The sulfur contents were not known and, therefore, the standard sulfur level of 0.07% was assumed. The mean deviation between x-ray and polarographic methods is ±0.05 ml. of tetraethyllead per gallon while the maximum deviation is 0.16.

Unfortunately, evaporation prevented a check on the two samples with deviations of 0.14 and 0.16.

Determinations are run by one operator in under 7 minutes. Of the 7 minutes, about 3 are taken up with counting and the remaining with filling the cell, cleaning, computation, etc. This time can be reduced by introducing several cells and by attention to some of the mechanical features of sample handling.

No extraction error or other uncertainties are introduced by chemical treatment or manipulation of the sample. This is a decided advantage of the x-ray method which, coupled with its speed and cheapness, makes it very attractive in those cases where it can be applied.

Table VII. Summary of Possible Errors

Source of Error	Conditions	Apparent Effect, Ml. TEL/Gal. Auto gas	Aviation gas
C/H ratio	$\Delta r = 0.2$	0.016	0.013
Sulfur	68.3% of samples 95.5% of samples	0.04 0.08	$\binom{0.035}{0.070}$ max.
Density	$\Delta \rho = 0.001$	0.01	0.01
Cell expan.	$\Delta T = 30°$ F.	0.004–0.02	0.004–0.02
Statistical	68.3% of samples 95.5% of samples	0.025 0.050	0.021 0.042
TEL mix	Aging	Probably small	
Sampling	Evaporation	Large if not controlled	

Table VIII. Comparison of X-Ray and Polarographic Methods for the Determination of Tetraethyllead in Automotive Gasolines

X-Ray	Polaro-graph	Difference	X-Ray	Polaro-graph	Difference
1.99	1.99	0.00	2.51	2.49	+0.02
2.68	2.69	−0.01	1.63	1.73	−0.10
1.40	1.45	−0.05	1.17	1.27	−0.10
1.51	1.45	+0.06	2.36	2.26	+0.10
1.62	1.56	+0.06	1.46	1.51	−0.05
0.00	0.00	0.00	1.71	1.73	−0.02
2.71	2.69	+0.02	2.72	2.64	+0.08
1.50	1.50	0.00	2.97	2.96	+0.01
0.38	0.40	−0.02	3.16	3.08	+0.08
1.79	1.77	+0.02	0.08	0.00	+0.08
2.41	2.37	+0.04	0.00	0.00	0.00
1.51	1.58	−0.07	0.54	0.70	−0.16
2.92	3.06	−0.14			

Mean deviation ±0.05 ml. TEL/gal.
Sulfur content assumed to be 0.07%; single determinations.

COMPARISON OF METHODS

The chemical method (A.S.T.M. D 526–48T), the polarographic methods, and the x-ray absorption methods are the principal ones in use today for tetraethyllead determinations. These are compared below with respect to speed, accuracy, and cost. In each case, the data refer to operations in the authors' laboratories and are not necessarily representative of industry averages.

Elapsed time, shown in the second column of Table IX, is defined as the interval between the start of an analysis and its completion under normal routine operation involving the number of samples which one operator can handle conveniently in one working day. Particularly in the case of polarographic determinations, this depends upon the established customs of each laboratory. "Rush" samples can frequently be reported in shorter periods. In some laboratories, the elapsed chemical time exceeds 24 hours. Operator time is the average of that which is devoted to each sample.

The accuracy of the chemical method, quoted from the A.S.T.M. D-2 manual (3), is based upon cooperative work. The polarographic and x-ray figures are estimates based upon careful comparisons with the chemical method of a large number of samples typical of current production. As discussed above, uncontrollable errors may enter into the x-ray method, chief of

which is the unknown sulfur content of the base stock. In blending operations and in other cases where the base stock is constant or the sulfur content is known, the accuracy can be expected to average 0.03 ml. of tetraethyllead per gallon. The effect of variations in the base stock itself, as reflected by its carbon-hydrogen ratio, is slight.

Table IX. Comparison of Chemical, Polarographic, and X-Ray Methods for Determination of Tetraethyllead in Gasoline

Method	Speed			Accuracy, Ml. TEL/Gal.	Relative Cost	
	Elapsed time	Oper-ator time	Samples per day		Equip-ment	Oper-ator time
	Hours	Min.				
Chemical	24	40	12	0.04-0.06	1	5.7
Polarograph	1-4	30	16	0.05	10	4.3
X-ray	0.12	7	60	0.10	15	1.0

In the last two columns of Table IX are given approximate values of the relative costs of the three methods. Chemical equipment is taken as $200. In addition, some fraction of the cost of an analytical balance should be added to this charge. Polarographic and x-ray equipment runs about $2000 and $3000, respectively.

CONCLUSION

In transferring from the chemical method to the x-ray method for the determination of tetraethyllead in gasoline, with samples handled at the rate of 2000 per year, it is conservatively estimated that the saving in personnel time is "paying out" the equipment cost every 6 months. In comparison, with the polarographic method, the x-ray method pays out the equipment every 8 months. A nontechnical operator with no previous x-ray experience is now handling all samples.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the advice received from J. B. Rather, Jr., J. H. Buck, and R. W. Moore, under whose direction this work has been done. The authors also wish to thank George Kokotailo, Robert Murphy, Harold Sobcov, Paul

Weisz, and John Wilczewski for assistance in collecting the data and developing the methods reported in this paper.

LITERATURE CITED

(1) Aborn, R. H., and Brown, R. H., IND. ENG. CHEM., ANAL. ED., 1, 26 (1929).
(2) Allen, S. J. M., "Handbook of Chemistry and Physics," 30th ed., Cleveland, Ohio, Chemical Rubber Publishing Co., p. 2005, 1947.
(3) American Society for Testing Materials, Philadelphia, Pa., "A.S.T.M. Standards on Petroleum Products and Lubricants," 1949.
(4) Anon., Instruments, 22, 69 (1949).
(5) Birks, L. S., Brooks, E. J., Friedman, H., and Roe, R. M., ANAL. CHEM., 22, 1258 (1950).
(6) Blade, O. C., U. S. Bur. Mines, Rept. Invest. 4567 (July 1949).
(7) Borup, R., and Levin, H., Am. Soc. Testing Materials, Proc., 47, 1010 (1947).
(8) Calingaert, G., Lamb, F. W., Miller, H. L., and Noakes, G. E., ANAL. CHEM., 22, 1238 (1950).
(9) Chesley, F. G., Rev. Sci. Instruments, 14, 3 (1943).
(10) Compton and Allison, "X-Rays in Theory and Experiment," New York, D. Van Nostrand Co., 1936.
(11) Doxey, G. A., Electronics, 22, 87 (December 1949).
(12) Engström, A., Acta Radiol. Suppl. 63 (1946); Rev. Sci. Instruments, 18, 681 (1947).
(13) Frediana, H. A., and Bass, L. A., Oil Gas J., 39, 51 (1940).
(14) Hevesy, G. von, "Chemical Analysis by X-Rays and Its Applications," New York, McGraw-Hill Book Co., 1932.
(15) Liebhafsky, H. A., ANAL. CHEM., 21, 17 (1949).
(16) Liebhafsky, H. A., Smith, H. M., Tanis, H. E., and Winslow, E. H., Ibid., 19, 861 (1947).
(17) Middel, H. D., "Four New Methods of Industrial Gas Analysis," Atomic Energy Commission, Pub. MDDC-855 (April 11, 1947).
(18) Moriarty, C. D., Gen. Elec. Rev., 50, 39 (February 1947).
(19) Pavelka, F., and Gentile, G., Mikrochemie ver. Mikrochim. Acta, 33, 152 (1947).
(20) Sproul, "X-Rays in Practice," New York, McGraw-Hill Book Co., 1946.
(21) Sullivan, M. V., and Friedman, H., IND. ENG. CHEM., ANAL. ED., 18, 304 (1946).
(22) Victoreen, J. A., J. Applied Phys., 19, 855 (1948).
(23) Ibid., 20, 1141 (1949).
(24) Victoreen, J. A., private communication, 1949.
(25) Vollmar, R. C., Petterson, E. E., and Petruzzelli, P. A., ANAL. CHEM., 21, 1491 (1949).
(26) Winslow, E. H., Smith, H. M., Tanis, H. E., and Liebhafsky, H. A., Ibid., 19, 866 (1947).
(27) Zemany, P. D., Winslow, E. H., Poellmitz, G. S., and Liebhafsky, H. A., Ibid., 21, 493 (1949).

Tetraethyllead in Gasoline

X-Ray Fluorescence Analysis of Ethyl Fluid in Aviation Gasoline

L. S. BIRKS, E. J. BROOKS, HERBERT FRIEDMAN, AND R. M. ROE

U. S. Naval Research Laboratory, Washington, D. C.

THE application of x-ray methods to chemical analysis has received attention during the past few years as a result of the development of improved techniques for intensity measurements with Geiger counters, and with the combination of photomultiplier tube and phosphor. Several articles have dealt with the particular problem of the determination of tetraethyllead in gasoline by x-ray absorption (1, 3, 4). The present paper describes the utilization of the x-ray fluorescence spectrum for the determination of lead and bromine in gasoline. In contrast to the simple absorption measurement, the fluorescence analysis

can be applied without independent knowledge of the composition of the base stock or variations in the composition of the ethyl fluid. Under conditions established for routine analysis, it should be possible to make from ten to twenty determinations per hour of both lead and bromine with an accuracy comparable to that achieved by conventional chemical methods. The measurements described here were undertaken with the purpose of evaluating the possibilities of the fluorescence method and do not constitute examples of typical results obtained in routine analyses. For routine purposes, specialized instrumentation can be designed to provide much more efficient performance.

X-ray fluorescence analysis was applied to the quantitative determination of lead and bromine in aviation gasoline. The characteristic x-ray lines used were the lead $L\alpha$ line of wave length 1.17 A. and the bromine $K\alpha$ line at 1.04 A. A 1-minute count at the peak of the lead line gave a probable error of ±0.06 ml. per gallon in a content of 4 ml. of tetraethyllead per gallon of gasoline; for the same counting interval, the bromine line could be measured with a probable error of ±0.16 ml. per gallon in 1.8 ml. of ethylene bromide per gallon of gasoline. The statistical accuracy improves in proportion to the square root of the length of the counting period. The relative intensities of the lead and bromine lines determined the relative amounts of the two present independently of variations in the base stock. Presence of additives such as chlorine had a negligible effect on the determination of lead and bromine.

EXPERIMENTAL

The x-ray fluorescence analysis apparatus used in this work was the same as that described by Friedman and Birks (2), and is shown schematically in Figure 1.

A sealed-off x-ray tube of the type employed in x-ray diffraction studies is the primary x-ray source. The primary radiation enters the liquid specimen, which is contained in a plastic cell, through a thin cellophane window. Characteristic fluorescent x-rays, excited within the sample, radiate in all directions. A portion of the fluorescent radiation emerges through the cellophane window in a direction defined by a collimator consisting of small-diameter tubings. The essentially parallel rays that pass through this collimator are analyzed by a single crystal spectrometer. At particular angles, the crystal is in position to reflect the characteristic wave length of a bromine x-ray fluorescence line, and the peak intensity, which is measured by a Geiger counter, is directly related to the atomic concentration of that element in the specimen.

In contrast to the requirements for absorption analysis, where the mass of the specimen must be accurately defined, the depth of the cell used in the fluorescence analysis has no influence on the results, provided that it exceeds a minimum. This minimum thickness is the greatest depth from which an appreciable intensity of fluorescent rays can emerge through the cellophane window.
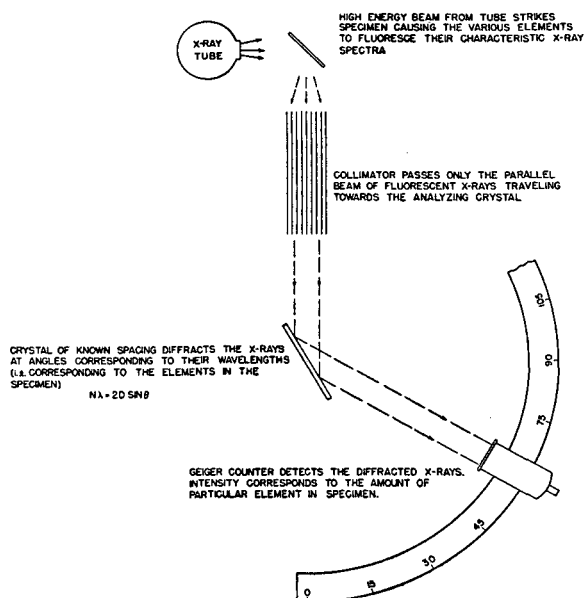
The characteristic x-ray lines which are utilized in the present analysis are the $L\alpha$ of lead at 1.17 A. and the $K\alpha$ of bromine at 1.04 A., as shown in Figure 2. Using a fluorite crystal as the analyzer, the Pb $L\alpha$ line falls at a Bragg angle of 17.7° and Br $K\alpha$ at 15.6°. Although these two lines are sufficiently well separated so that there is no difficulty in resolving them, there is a slight overlapping of the much weaker $L\beta_4$ line of lead at 15.1° with Br $K\alpha$. The Pb $L\beta_4$ line contributes to the background on the low angle side of the bromine line by an amount proportional to the lead content of the sample. Figure 3 illustrates the lead and bromine lines obtained with ethyl fluid in 2-methylheptane (iso-octane) for several concentrations.
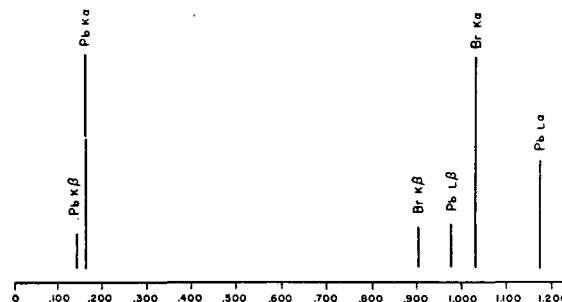

Figure 2. Wave Lengths of Characteristic X-Ray Lines of Lead and Bromine Spectra

Of primary importance to the analysis is the effect of variations in composition of the tetraethyllead and base stock on the measured intensities of lead and bromine x-ray lines. Figure 4 shows the line intensity versus concentration of bromine for ethylene dibromide and ethyl fluid in 2-methylheptane. The curve for the ethyl fluid indicates a lower intensity for a given bromine concentration because of the absorption of the fluorescent bromine rays by the lead that is present in the fluid. In Figure 5 similar data are given for the lead line for tetraethyllead and ethyl fluid in 2-methylheptane. Because the reduction in intensity of either the lead or bromine line in ethyl fluid is dependent on the relative amount of the other components present in the sample, quantitative analysis based on the measurement of only one element is possible only if the concentration of the second component is known. However, the determination of both lead and bromine is possible if both lines are measured and their relative intensities are referred to a series of calibration curves based on different ratios of the two elements. Because the spread of the pairs of curves shown in Figures 4 and 5 for the extreme
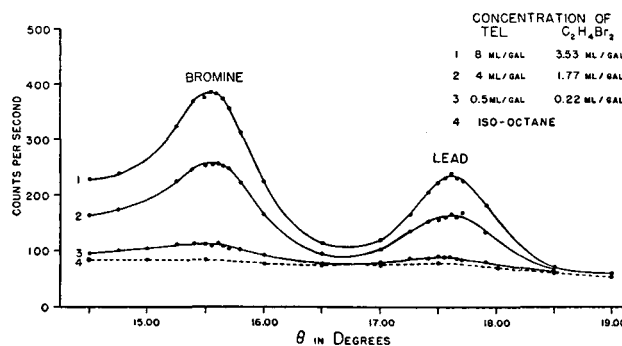

Figure 1. Schematic Representation of X-Ray Fluorescence Analysis Method

Figure 3. Spectral Lines of Lead and Bromine

Obtained with x-ray fluorescence spectrometer. 40 kv., 15 ma., Mo target

cases is relatively small, only a few intermediate ratios need be calibrated to permit accurate determinations of both lead and bromine independently of any composition of ethyl fluid likely to be encountered.

ACCURACY OF X-RAY INTENSITY MEASUREMENTS

Assuming a constant intensity of primary x-rays and no spurious pulses in the counting system, a statistical accuracy can be assigned to any determination of the intensity of an x-ray line. This accuracy depends then, only on the total count, N_S, of the line plus background and on the background count, N_B, alone. In the absence of background the probable error of the count on the x-ray line characteristic of the element being analyzed is simply $^2/_3(N_S)^{1/2}$. In the measurements illustrated in Figure 3, the intensity at the peak of the lead line for 4 ml. of tetraethyllead per gallon of 2-methylheptane was about 85 counts per second above background. At the end of 1 minute of counting, N_S would total 5100 counts with a relative probable error of $^2/_3(5100)^{-1/2}$ or 0.9% if no background were present. The background, however, was also 5100 counts per minute, as intense as the characteristic line, and its probable error combined with that of the count on the fluorescence line according to

$$\text{P.E. } (N_S - N_B) = {}^2/_3[(N_S) + (N_B)]^{1/2} \tag{1}$$
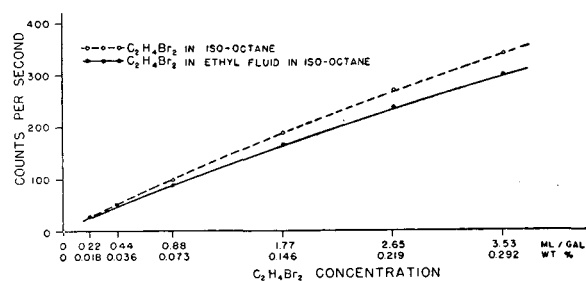
Figure 4. Intensity of Br $K\alpha$ vs. Concentration of Bromine from Ethylene Dibromide and Ethyl Fluid in 2-Methylheptane

Intensity of bromine line from ethyl fluid is lowered by partial absorption of bromine radiation by lead content of ethyl fluid.
40 kv., 15 ma., Mo target

In this example, N_S is about equal to $2N_B$, and the relative probable error of the difference, $N_S - N_B$, becomes approximately

$$\text{Rel. P.E. } (N_S - N_B) = {}^2/_3 \frac{(3N_B)^{1/2}}{N_S - N_B} = \frac{2}{\sqrt{3N_B}} \tag{2}$$

which is about 1.6%. The statistical accuracy of the counting measurement of the intensity of lead radiation was therefore ±1.6% of 4 ml. per gallon or ±0.06 ml. per gallon, when counting was carried out for 1 minute. Similar considerations apply to the measurement of Br $K\alpha$ intensity. Under identical conditions, the bromine line intensities were approximately four times those of lead for equal volume concentrations of ethylene dibromide and tetraethyllead in 2-methylheptane.

The relative probable error of any counting measurement of an x-ray line decreases in proportion to the reciprocal of the total count. Accordingly, to improve the accuracy of the above results by a factor of 10 would require counting for 100 minutes as compared to 1 minute. The practicality of long counting periods is governed by the ability to eliminate errors arising from fluctuations in primary x-ray intensity. Several systems of x-ray tube voltage and current stabilization have been described, but perhaps the simplest method for the present purpose is to monitor the primary x-ray beam with an auxiliary counting system. By making the counting time conform to a fixed dose

measured by the monitor, it is possible to allow wide fluctuations in primary x-ray intensity without affecting the accuracy of the measurement of the fluorescence count. Details of this technique will be described shortly.

The most serious limitation on the sensitivity of analyses such as those described here is the presence of the background radiation upon which the characteristic lines are superimposed. For example, if the background is equal to the line, as in the above example for 4 ml. of tetraethyllead per gallon of 2-methylheptane, six times as many counts are required to obtain any given accuracy as would be the case if the background were not present. Almost all the background in the x-ray spectrum can be attributed to scattering of the primary radiation by the liquid sample. The intensity of such scattered radiation depends on the nature of the liquid sample and is generally stronger, the greater the hydrogen content of the liquid. However, the small variation of hydrogen content in "base stocks" such as 2-methylheptane, benzene, cyclohexane, and aviation base stock is not enough to affect the background intensity appreciably. The background problem is aggravated if the characteristic lines of the x-ray tube target fall in the same spectral region as those of the sample to be analyzed. For, after being scattered by the sample, these primary spectrum rays are indistinguishable from the fluorescent rays and reach the Geiger counter in the same way. A molybdenum target is much to be preferred over tungsten, which has a multiplicity of L series lines in the region of the lead $L\alpha$. The molybdenum $K\alpha$ and $K\beta$ lines occur at wave lengths shorter than 0.7 A. and do not interfere with either the lead or bromine analysis. At the same time, they are particularly effective in exciting the bromine fluorescence spectrum.

POSSIBILITIES FOR IMPROVING EFFICIENCY OF X-RAY ANALYSIS

There are possibilities for improving the counting rates shown here with minor modifications in the equipment. The dependence of Geiger tube efficiency on wave length has been discussed in earlier publications. In the spectral region about 1 A., the atmospheric pressure argon-filled counter, which was used here, was about 50% efficient in producing a count for each quantum entering the counter. An additional 25% of the entrant quanta could be counted by merely doubling the length of the counter, because the x-ray absorption takes place almost entirely in the gas path in the direction of the axis of the tube. A second possibility of increasing counting rate is to utilize greater capacity in the x-ray tube. The Machlett AEG-50T type of tube could be used in the present apparatus if provided with a molybdenum target. This would permit utilization of double the present x-ray output. Currently manufactured x-ray diffraction power supplies are capable of supplying the full 50 ma. at 50 kv. that a tube of the AEG-50T type is rated for. Finally, a considerable improvement in counting rate would be derived by designing an x-ray tube specifically for the fluorescence spectrometer, in which
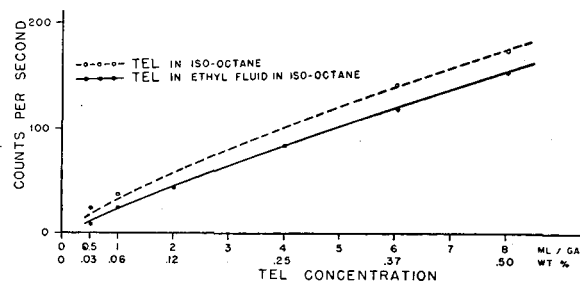
Figure 5. Intensity of Pb $L\alpha$ vs. Concentration of Lead from Tetraethyllead and Ethyl Fluid in 2-Methylheptane

Intensity of lead line from ethyl fluid is lowered by partial absorption of lead radiation by bromine content of ethyl fluid

the distance of target to specimen could be reduced to perhaps one third that required by the tube used here. This would permit a ninefold gain in intensity.

The geometry of the simple arrangement shown in Figure 1 can readily be altered to accommodate several specimens simultaneously, if desired. A four-window x-ray tube can obviously serve four samples simultaneously. After the spectrum is once established as shown in Figure 3, measurements need be made at the peak and background positions only, and the spectrometer for each specimen can be reduced to simply a crystal, a collimator, and a Geiger counter set for only two fixed angular relationships to each other.

CONCLUSION

The fluorescence method is capable of rapid determination of both lead and bromine in gasoline with an accuracy comparable to the best methods available at present. No special sample preparation is required for the analysis, which can be performed by relatively unskilled personnel. Although the results described in this paper were obtained with "aviation mix" type of ethyl

fluid which normally contains only tetraethyllead, ethylene dibromide, and a few per cent of kerosene, dye, and other impurities, the determination of lead and bromine would be just as satisfactory if applied to a "motor mix" containing ethylene dichloride in addition, inasmuch as the presence of chlorine was found experimentally to have a negligible effect on the background intensity of the lead and bromine lines. The presence of sulfur, which introduces difficulties in absorption analyses, does not contribute any interfering lines in the spectral region of lead and bromine fluorescence. It is entirely feasible to consider the adaptation of the fluorescence method to semiautomatic plant control problems, as well as laboratory analyses.

LITERATURE CITED

(1) Aborn and Brown, Ind. Eng. Chem., Anal. Ed., 1, 26 (1929).
(2) Friedman and Birks, Rev. Sci. Instruments, 19, 323 (1948).
(3) Sullivan and Friedman, Ind. Eng. Chem., Anal. Ed., 18, 304 (1946).
(4) Zemany, Winslow, Poellmitz, and Liebhafsky, Anal. Chem., 21, 493 (1949).

Received March 22, 1950.

[End of Symposium]

Determination of *trans*-Octadecenoic Acids, Esters, and Alcohols in Mixtures

Infrared Spectrophotometric Method

O. D. SHREVE AND M. R. HEETHER, *Philadelphia Laboratory, E. I. du Pont de Nemours & Company, Inc., Philadelphia 46, Pa.*, AND H. B. KNIGHT AND DANIEL SWERN, *Eastern Regional Research Laboratory, Philadelphia 18, Pa.*

An infrared spectrophotometric method, based on differences in absorption at 10.36 microns, is described for determination of *trans*-octadecenoic acids, esters (including glycerides), and alcohols in the presence of the corresponding cis and saturated compounds. Extinction coefficients at 10.36 microns are reported for seventeen pure cis and trans monounsaturated and saturated acids, esters, and alcohols. The method is rapid, accurate, and directly applicable to determination of trans isomers in acid, ester, or alcohol mixtures. Only small samples are required, and they can be recovered. The authors know of no other method for determination of trans alcohols or for direct determination of trans esters in mixtures, although approximate and time-consuming chemical methods are available for the acids. The accuracy and precision of the method are indicated from analyses of nineteen synthetic mixtures of known composition. The infrared method is suggested as a valuable tool to investigators conducting research on oxidation, isomerization, polymerization, composition, and hydrogenation of fats and their components and derivatives, and on the preparation of pure unsaturated acids and esters.

THE two methods now employed for determining trans components in acid or ester mixtures are not based on any unique characteristic of trans compounds, but either on the preferential insolubility of lead (*1, 22, 30*) or other salts (*5, 8, 9, 16, 27, 29*) of trans acids in organic solvents, or on the difference between the equilibrium constants of cis and trans acids in their reaction with iodine in carbon tetrachloride solution (*4, 21*). These chemical methods are generally laborious and time-consuming, they require large quantities of sample, and they are of doubtful reliability in many applications (*25*). The authors know of no previously reported method for determination of trans alcohols in mixtures.

Published infrared absorption spectra (*17, 19*) for pure elaidic (*trans*-9-octadecenoic), petroselaidic (*trans*-6-octadecenoic), and vaccenic (*trans*-11-octadecenoic) acid, methyl elaidate and petroselaidate, elaidyl alcohol (*trans*-9-octadecenol), and trielaidin

show a strong absorption maximum at 10.36 microns, which is absent in the corresponding cis and saturated compounds. No marked differences exist, however, between corresponding trans, cis, and saturated compounds in other regions of the infrared spectrum.

Although several groups of workers have reported that olefins with an internal unsubstituted double bond (R_1—CH=CH—R_2) have an absorption band at about 10.36 microns (*7, 11, 13, 14, 28*), Rasmussen, Brattain, and Zucco (*18*) were apparently the first to point out that this absorption is due to trans configuration of this group. Absorption at this wave length has since been employed for both the qualitative and quantitative analyses of a variety of materials (*2, 3, 10, 11, 14, 15*), but not for the quantitative determination of *trans*-octadecenoic acids, esters, and alcohols in mixtures. An infrared spectrophotometric method based on the difference at 10.36 microns has been developed by the authors for

this type of determination. In developing the method, extinction coefficients were determined on the compounds mentioned above as well as on pure oleic (cis-9-octadecenoic), petroselinic (cis-6-octadecenoic), palmitic (hexadecanoic), and stearic (octadecanoic) acids, methyl oleate and stearate, triolein, trimyristin (glyceryl tritetradecanoate), and oleyl (cis-9-octadecenol) and stearyl (octadecanol) alcohols. In addition, mixed palmitostearins were obtained by crystallization of completely hydrogenated vegetable oil triglycerides. The infrared method is rapid, specific, and accurate, it is directly applicable to determination of trans components in acid, ester, or alcohol mixtures, only small samples are required, and the sample can be recovered.

APPARATUS, MATERIALS USED, AND PROCEDURE

Apparatus. This has been reported (25).

Materials Used. The methods of preparation of most of the reference compounds have been described (25). Oleyl alcohol, boiling point 180.1–180.4° C. at 3.6 mm., n_D^{30} 1.4562, and iodine number 93.7 (calculated 94.5), was prepared by repeated fractional distillation and low temperature crystallization of a good commercial grade (24). Elaidyl alcohol, melting point 35.7–35.9° and iodine number 93.7, was prepared by isomerization of oleyl alcohol with powdered selenium at 220–225° (23). Stearyl alcohol, melting point 58°, was obtained by recrystallization of the purest commercial grade three times from 95% ethyl alcohol and once from methanol at 0° (7 to 10 ml. of solvent per gram of solute).
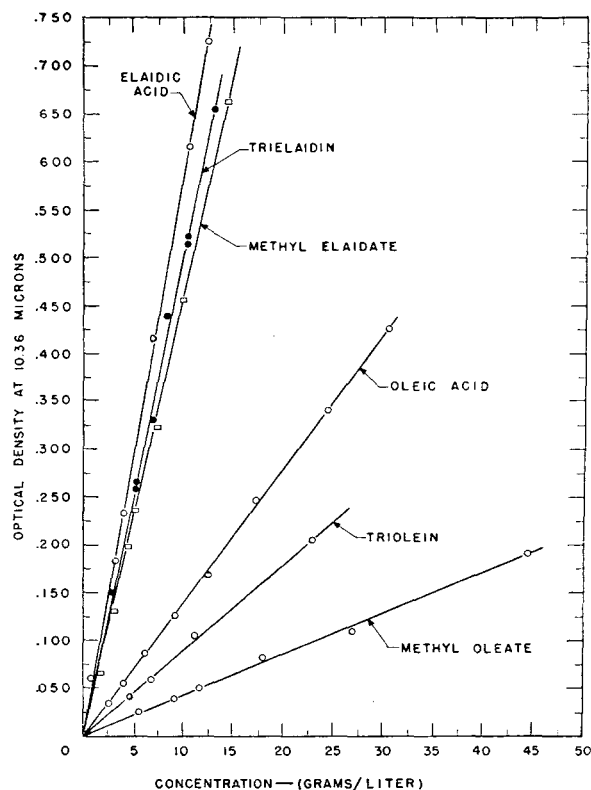
Figure 1. Optical Density at 10.36 Microns as Function of Concentration in Carbon Disulfide

Determination of Extinction Coefficients of Pure Compounds. The procedure employed has been described (25). Concentration ranges covered were 0 to approximately 15 grams per liter for all trans compounds (strong absorbers); 0 to approximately 30 grams per liter for the cis monounsaturated and saturated acids, glycerides, and alcohols (moderate to weak absorbers); and 0 to approximately 45 grams per liter for the cis monounsaturated and saturated methyl esters (extremely weak absorbers). Plots of optical density as a function of concentration in grams

per liter showed excellent adherence to Beer's law over the concentration range studied. Figure 1 shows some representative plots. The extinction coefficients, k, to be used in analysis were obtained by dividing the slopes of the straight-line Beer's law plots (on an expanded scale) by the cell thickness (20) in centimeters. Thus $k = \dfrac{\text{optical density at } 10.36\mu}{(\text{concn., grams/liter})(0.1054 \text{ cm.})}$. Table I lists the k values and values for the molar extinction coefficient, ϵ ($k \times$ molecular weight).

Table I. Extinction Coefficients at 10.36 Microns for Pure cis and trans Monounsaturated and Saturated Acids, Methyl Esters, Triglycerides, and Alcohols

Compound	Extinction Coefficients k^a	ϵ^b	No. of Determinations
Oleic acid	0.133	37.6	8
Petroselinic acid	0.129	36.4	7
Palmitic acid	0.129	33.1	7
Stearic acid	0.123	35.0	7
Elaidic acid	0.552	155.9	7
Petroselaidic acid	0.560	158.2	5
Methyl oleate	0.041	12.2	7
Methyl stearate	0.028	8.4	7
Methyl elaidate	0.442	131.1	7
Methyl petroselaidate	0.454	134.6	6
Triolein	0.084	74.4	4
Trimyristin	0.087	62.9	5
Palmitostearins	0.078	...	7
Trielaidin	0.475	420.6	8
Oleyl alcohol	0.069	18.5	6
Stearyl alcohol	0.058	15.7	6
Elaidyl alcohol	0.523	140.4	6

^{a} $k_{10.36} = \dfrac{\text{optical density at } 10.36\mu}{(\text{concn., grams/liter}) \ (0.1054 \text{ cm.})}$

^{b} $\epsilon_{10.36} = \dfrac{\text{optical density at } 10.36\mu}{(\text{concn., moles/liter}) \ (0.1054 \text{ cm.})}$

Analysis of Known and Unknown Mixtures. A weighed sample of the mixture is diluted to 10 ml. with carbon bisulfide in a volumetric flask, and the optical density of the solute at 10.36 microns is determined under conditions identical with those described above for the pure compounds. In general, the total concentration of the mixture should be adjusted to give a solute optical density between 0.2 and 0.6. The concentration selected will depend on the approximate content of trans component expected and whether an acid, methyl ester, glyceride, or alcohol mixture is being analyzed. For mixtures containing substantial percentages of trans component, 0.1 to 0.2 gram diluted to 10 ml. will usually give satisfactory results. Higher total concentrations are employed when the trans content of the mixture is low. In any case the sample can be greatly reduced by semimicro weighing and dilution techniques.

Calculation of Results. The following formulas are used in calculating the weight per cent of trans component in mixtures:

MIXTURES CONTAINING cis- AND trans-OCTADECENOIC COMPONENTS ONLY.

$$\text{trans component, weight } \% = \frac{100 \ (k_{ob} - k_c)}{k_T - k_c} \quad (1)$$

where k_{ob} = "observed extinction coefficient" for the mixture = $\dfrac{\text{optical density at } 10.36\mu}{(\text{total concn., grams/liter}) \ (\text{cell thickness, cm.})}$

k_c = extinction coefficient of pure cis compound (Table I)
k_T = extinction coefficient of pure trans compound (Table I)

MIXTURES CONTAINING trans-OCTADECENOIC AND SATURATED COMPONENTS ONLY.

$$\text{trans component, weight } \% = \frac{100 \ (k_{ob} - k_s)}{k_T - k_s} \quad (2)$$

where k_s is the extinction coefficient of the pure saturated compound (Table I).

Mixtures Containing *cis*- and *trans*-Octadecenoic and Saturated Components.

$$\text{trans component, weight } \% = \frac{100\,(k_{ob} - k_c Y - k_s Z)}{k_T - k_c} \quad (3)$$

where the k values are as defined above; Y is the total weight fraction of octadecenoic components (cis plus trans), and Z is the weight fraction of saturated components. Y is calculated from the iodine value of the mixture and Z is obtained by difference.

In practical applications to unknown mixtures, the *cis*- and *trans*-octadecenoic fractions may each consist of one or more compounds in which the exact position of the double bond is unknown. Also the relative proportions of long-chain saturated compounds in the saturated fraction are often unknown. Therefore, the following facts, evident from the data of Table I, are fortunate.

Shifting the double bond from the Δ^9 to the Δ^6 position in either the *cis*- or *trans*-octadecenoic acids produces only a small change in extinction coefficient. The same holds for the *trans*-methyl esters, and therefore might be expected to hold for the *cis*-methyl esters, and the *cis*- and *trans*-glycerides and alcohols. Furthermore, it seems safe to assume that an equivalent shift in the opposite direction to Δ^{12} or to any intermediate position would likewise produce little change. (The effect of more drastic shifts remains to be investigated.)

The extinction coefficients for the various *cis*-octadecenoic and saturated acids studied are all approximately the same. This relation also holds for the cis and saturated glycerides and alcohols and to a somewhat lesser degree for the cis and saturated methyl esters.

Table II. Analyses of Synthetic Mixtures for trans Component

Composition of Mixture, %[a]			trans Component Found, %			
ME	MO	MS	1	2	Av.	Difference
66.91	33.09	0	66.52	66.81	66.66	−0.25
49.03	50.97	0	49.52	49.10	49.31	0.28
3.12	96.63	0	3.20	3.38	3.29	0.17
26.12	23.87	50.01	25.47	25.68	25.58	−0.54
9.96	16.16	73.88	9.67	9.15	9.41	−0.55
3.17	15.00	81.83	3.42	3.28	3.35	0.18
EA	OA	SA				
67.48	32.52	0	67.11	67.52	67.32	−0.16
18.10	81.90	0	17.59	18.40	18.00	−0.10
10.41	89.59	0	10.52	11.11	10.82	0.41
3.31	96.69	0	3.50	3.80	3.65	0.34
65.17	20.11	14.72	66.21	65.77	65.99	0.82
39.44	32.28	28.28	38.33	38.61	38.47	−0.97
10.32	7.07	82.61	10.51	10.73	10.63	0.31
4.44	20.30	75.30	3.73	3.92	3.83	−0.61
EAL	OAL	SAL				
65.87	34.13	0	65.72	65.84	65.78	−0.09
50.13	49.87	0	50.22	49.98	50.10	−0.03
19.95	80.05	0	20.00	19.85	19.92	−0.03
19.48	39.22	41.30	19.07	19.41	19.24	−0.24
2.99	51.14	45.87	3.00	3.11	3.05	0.06

[a] ME = methyl elaidate; MO = methyl oleate; MS = methyl stearate; EA = elaidic acid; OA = oleic acid; SA = saturated acids; EAL = elaidyl alcohol; OAL = oleyl alcohol; SAL = stearyl alcohol.

In unknown acid mixtures that contain no octadecenoic acids outside the Δ^6 to Δ^{12} range, the total percentage of trans acids present can be calculated with acceptable accuracy by using the average k value of the two trans acids from Table I for k_T, that of the two cis acids for k_c, and that of the two saturated acids for k_s in the appropriate formula above. Results on unknown methyl ester, glyceride, or alcohol mixtures may be similarly calculated by using appropriate average or single values in Table I. In view of the above statement, determination of iodine number in a trans-cis-saturated mixture (of either of the four types) can be eliminated if desired, and results calculated from the following simplified formula:

$$\text{trans component, weight } \% = \frac{100\,(k_{ob} - k_{av.})}{k_T - k_{av.}} \quad (4)$$

where k_T is the appropriate average or single k value for trans compounds and $k_{av.}$ is the average k value for both cis and saturated compounds.

For given selected values of k_c and k_s, Formulas 3 and 4 will yield identical results (expressed as per cent trans, to the nearest 0.01%) when $Y = Z = 0.5$—that is, when total per cent octadecenoic components equal total per cent saturated components. As Y departs from 0.5 in either direction, the absolute error entailed by use of Formula 4 increases. In the most unfavorable cases likely to be encountered in analyzing acid mixtures ($Y = 0.03$ or $Y = 0.97$), this error is about 0.5% when the average value for the two trans acids is used for k_T, that of the two cis acids for k_c, that of the two saturated acids for k_s, and that of the latter for $k_{av.}$. In most cases, the error will be much less.

RESULTS

Table II gives the percentage of trans component obtained on nineteen known synthetic mixtures of pure compounds. The results show that in general the infrared method has a satisfactory degree of precision and accuracy. The best oleic acid from the chemical identity standpoint is that obtained from olive oil (6). Oleic acid obtained from animal fat sources (12, 24, 26) contains varying amounts of trans isomers (4 to 45%), depending on the starting material and the preparative process employed (25). This is probably not disadvantageous for many technical applications, but it suggests that these oleic acids should not be used when chemical homogeneity is important.

GENERAL COMMENTS

Because of the present state of infrared spectrophotometry, extinction coefficients reported in this paper cannot be employed directly by other workers. Variations in such factors as scattered radiation, wave-length calibration, slit-width settings, and accuracy of cell-thickness measurements make it necessary to determine the required extinction coefficients on the instrument being used under the exact conditions to be employed in the analysis. It may be unnecessary, however, to determine the extinction coefficients of all the compounds reported in Table I—for example, from redeterminations of one trans and one cis or saturated compound, in conjunction with relative values calculated from those in Table I, it should be possible to calculate sufficiently accurate values for the remaining compounds.

ACKNOWLEDGMENT

LITERATURE CITED

(1) American Oil Chemists' Society, "Official and Tentative Methods," 2nd ed., Official Method Cd 6-38, 1946.
(2) Anderson, J. A., and Seyfried, W. D., ANAL. CHEM., 20, 998 (1948).
(3) Barnes, R. B., Gore, R. C., Stafford, R. W., and Williams, V. Z., Ibid., 20, 402 (1948).
(4) Bertram, S. H., Oil Colour Trades J., 94, 1227 (Oct. 28, 1938).
(5) Bömer, A., and Hagemann, B., Fette u. Seifen, 50, 1 (1943).
(6) Brown, J. B., and Shinowara, G. Y., J. Am. Chem. Soc., 59, 6 (1937).
(7) Dinsmore, H. L., and Smith, D. C., ANAL. CHEM., 20, 11 (1948).
(8) Escher, H. H., Helv. Chim. Acta, 12, 103 (1929).
(9) Falciola, P., Gazz. chim. ital., 40 II, 217, 425 (1910).
(10) Field, J. E., Woodford, D. E., and Gehman, S. D., J. Applied Phys., 17, 386 (1946).
(11) Fred, M., and Putscher, R., ANAL. CHEM., 21, 900 (1949).
(12) Goebel, C. G., J. Am. Oil Chemists' Soc., 24, 65 (1947).
(13) Gore, R. C., and Johnson, J. L., Phys. Rev., 68, 283 (1945).
(14) Hampton, R. R., ANAL. CHEM., 21, 923 (1949).
(15) Hart, E. J., and Meyer, A. W., J. Am. Chem. Soc., 71, 1980 (1949).
(16) Meigen, W., and Neuberger, A., Chem. Umschau, 29, 337 (1922).
(17) Rao, P. C., and Daubert, B. F., J. Am. Chem. Soc., 70, 1102 (1948).
(18) Rasmussen, R. S., Brattain, R. R., and Zucco, P. S., J. Chem. Phys., 15, 135 (1947).

(19) Shreve, O. D., Heether, M. R., Knight, H. B., and Swern, D., paper presented before Division of Organic Chemistry at 116th Meeting, AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J., 1949.

(20) Smith, D. C., and Miller, E. C., J. Optical Soc. Am., 34, 130 (1944).

(21) Steur, J. P. K. van der, Rec. trav. chim., 46, 278, 409 (1927).

(22) Stillman, R. C., and Andrews, J. T. R., Oil & Soap, 14, 257 (1937).

(23) Swern, D., Jordan, E. F., and Knight, H. B., J. Am. Chem. Soc., 68, 1673 (1946).

(24) Swern, D., Knight, H. B., and Findley, T. W., Oil & Soap, 21, 133 (1944).

(25) Swern, D., Knight, H. B., Shreve, O. D., and Heether, M. R., J. Am. Oil Chemists' Soc., 27, 17 (1950).

(26) Swern, D., Scanlan, J. T., and Roe, E. T., Oil & Soap, 23, 128 (1946).

(27) Tandon, B. L., Indian Soap J., 14, 114 (1948).

(28) Thompson, H. W., and Torkington, P., Trans. Faraday Soc., 41, 246 (1945).

(29) Tsujimoto, M., J. Chem. Ind. (Japan), 23, 1007 (1920).

(30) Twitchell, E., J. Ind. Eng. Chem., 13, 806 (1921).

Polarographic Determination of Aluminum

Use of an Organic Reagent

HOBART H. WILLARD AND JOHN A. DEAN[1]

University of Michigan, Ann Arbor, Mich.

A new method for the polarographic determination of aluminum is based on the reduction of an aluminum di-o-hydroxyazo complex at −0.5 volt vs. the saturated calomel electrode in an acetate buffer of pH 4.6. The method is sensitive to 0.005 mg. of aluminum per 50 ml., and is generally applicable to steels, nonferrous alloys, and minerals with an accuracy of ±3% after removal of interferences at a mercury cathode or by fusion with sodium carbonate.

A SURVEY of the polarographic literature reveals the lack of a suitable method for the determination of aluminum in the presence of many elements.

Prajzler (7) studied the simultaneous estimation of the metals occurring in the hydrous oxide and alkaline sulfide groups of qualitative analysis. He obtained a fairly well defined wave for the reduction of aluminum in 0.05 M barium chloride as supporting electrolyte. Gull (2) and Heller and Zan'ko (3) published procedures for the determination of aluminum in magnesium alloys. These authors found it necessary to adjust the pH of the solution carefully in order to prevent the succeeding discharge of hydrogen from masking the diffusion current of the aluminum wave, and at the same time to prevent precipitation of the aluminum as hydrous oxide. A similar procedure was used by Ford and LeMar (1) to determine aluminum oxide in portland cement, although they found it necesssary to allow the prepared solutions to stand overnight before polarographing to effect complete separation from iron and to allow any aluminum oxide to redissolve. Because the half-wave potential of aluminum (−1.7 volts vs. S.C.E.) is more negative than the half-wave potentials of most other cations generally encountered together with aluminum, most of these cations will produce diffusion currents preceding the aluminum wave. Parks and Lykken (6) developed an indirect procedure for aluminum based upon the decrease in wave height of a 0.05% alcoholic solution of 8-quinolinol in an ammonia–ammonium chloride buffer of pH 9.8 as a result of the precipitation of aluminum oxinate. However, a waiting period of 1 hour is necessary to secure even a 90% complete precipitation. Therefore, it seemed desirable to attempt to find a different approach to the polarographic determination of aluminum.

Weissler and White (9) used di-o-hydroxyazo dyes in a quantitative determination of aluminum fluorometrically, and it is known that the azo group yields a well defined diffusion wave. Consequently, these dyes were tested polarographically in the

[1] Present address, Department of Chemistry, University of Tennessee, Knoxville, Tenn.

presence of aluminum. It was found that a very sensitive direct polarographic determination of aluminum could be made at relatively low values of applied voltage after removal of any interfering ions. The success of the method probably revolves about the cis-trans equilibrium existing about the azo group of these dyes.

APPARATUS AND REAGENTS

A Sargent polarograph, Model XI, was used to obtain the polarograms. Provision was made to keep the solutions at 25° ± 0.2° C. A saturated calomel electrode was used as a reference electrode with an agar–saturated potassium chloride salt bridge providing contact with the electrolyte. Air was removed by bubbling nitrogen, purified by passage through chromous chloride solution, through the solutions for 15 minutes.

pH measurements were made with a Beckman model G pH meter calibrated against a 0.0500 M solution of potassium acid phthalate (pH = 4.01).

A standard solution of aluminum, 1.00 ml. = 0.100 mg., was prepared by dissolving 1.760 grams of potassium aluminum sulfate crystals in distilled water, adding 8 ml. of 72% (12 N) perchloric acid, and diluting to 1 liter. A weaker standard solution of aluminum, 1.00 ml. = 0.0100 mg., was prepared by pipetting out 100 ml. of the above solution, adding 7 ml. of 72% perchloric acid, and diluting to 1 liter with distilled water.

Ammonium acetate or sodium acetate solution, 2 N, was prepared by dissolving 154 or 164 grams, repectively, of the c.p. salt in distilled water and diluting to 1 liter.

The dye solution, Pontachrome Violet SW (Color Index 169) which is the sodium salt of 5-sulfo-2-hydroxy-α-benzene-azo-2-naphthol, was prepared by dissolving 0.50 gram of the dye in 1 liter of water.

EXPERIMENTAL

Two dyes were investigated: Pontachrome Violet SW and Pontachrome Blue Black R; the latter is the naphthalene analog of the former dye. Solutions of both dyes exhibited similar characteristics toward aluminum ions, but the Violet SW is more suitable, principally because of its greater solubility, and therefore this paper is confined essentially to the work done with Pontachrome Violet SW. In Figure 1 are shown the

current voltage curves of the dye alone and in the presence of aluminum ions. The total diffusion current remains unaltered but part of the wave is displaced approximately 0.2 volt more negative than the original dye wave and now appears as a distinct second wave.

Table I. Diffusion Current Constant of Aluminum-Pontachrome Violet SW Compound

(Supporting electrolyte, 0.2 N sodium acetate and sufficient perchloric acid to adjust pH to 4.7. Diffusion currents were measured at 25.0° C., and correction applied for diffusion current of unreacted dye. $m^{2/3}t^{1/6}$ = 2.31)

Aluminum, Mg./50 Ml.	i_d, Ma.	$\dfrac{i_d}{Cm^{2/3}t^{1/6}}$	i_d (Corrected)[a], $Cm^{2/3}t^{1/6}$
0.0125	0.165	7.70	5.36
0.0250	0.256	6.00	4.80
0.0500	0.495	5.75	5.17
0.0750	0.660	5.15	4.75
0.100	0.892	5.20	4.90
0.150	1.39	5.40	5.20
0.200	1.82	5.30	5.19
0.250	2.21	5.15	5.04
0.300	2.64	5.15	5.05
0.350	3.17	5.28	5.19
		Av.	5.09 ± 0.16

[a] Contains correction for observed zero current of 0.050 microampere.

Next a series of solutions was prepared which contained varying amounts of aluminum and excess dye. The supporting electrolyte consisted of a 0.2 M solution of sodium acetate adjusted to a pH of 4.7 with perchloric acid. Column 3 of Table I gives the diffusion current constants as calculated from the observed diffusion currents. It would appear that the diffusion current is proportional to the aluminum concentration only for moderate amounts of aluminum. However, if the measured diffusion currents are plotted versus the aluminum concentrations, as has been done in Figure 2, a linear relationship is obtained. Insolubility of the dye limits the amount of aluminum that can be determined to less than about 0.350 mg. in a volume of 50 ml. Inspection of the graph in Figure 2 reveals that the curve intersects the current axis at a value somewhat greater than zero and thus this would account for the discrepancies previously observed in the diffusion current constants. A similar zero current of almost equal magnitude has been reported by Volpi (8) for azobenzene. If the diffusion current constants are recalculated after subtraction of the zero current from the observed diffusion currents, as has been done for the values listed in column 4 of Table I, satisfactory agreement is obtained.

It remains to study the effect of such variables as pH, amount of diverse ions tolerated, and the temperature and time of standing.

Effect of pH. A series of solutions was prepared which contained 0.020% Pontachrome Violet SW and 0.250 mg. of aluminum per 50 ml. The solutions were 0.2 N in sodium chloroacetate or in sodium acetate. Sufficient sodium hydroxide solution or perchloric acid was added to adjust the pH to desired values. All pH measurements were made with the laboratory model Beckman pH meter. The solutions were allowed to attain equilibrium before any measurements were made.

The data obtained are plotted in Figure 3. The half-wave potentials of both portions of the diffusion current for the two waves are a linear function of pH, and can be represented by these empirical equations: for the first wave due to unreacted Violet SW, $E_{1/2} = 0.021 - 0.069$ pH; and for the second wave due to the aluminum-dye compound, $E_{1/2} = -0.258 - 0.058$ pH.

The hydrogen ion concentration also affects the magnitude of the diffusion current constant for the second part of the double wave, as shown graphically in Figure 4.

Like results obtained by Weissler and White (9), a rather abrupt cut-off is noticed for pH values less than about 3.2; however, the rapid increase in the magnitude of the diffusion

current constant above pH of 5 finds no parallel in fluorometric work and may be due to the discharge of the dye anion. The optimum pH range for polarographic work is 4.6 ± 0.1.

Time of Standing and Effect of Temperature. It was found that the diffusion currents of both portions of the combined waves were altered upon standing. The diffusion current of the first wave decreased, while the diffusion current of the second wave correspondingly increased. At room temperature the solutions required over 4 hours to reach final equilibrium. To determine whether the equilibrium diffusion current could be obtained in a shorter time, various solutions were placed in a water bath at temperatures exceeding 50° C. for a 5-minute period, and then were cooled to 25° C. before measurements were made. This step was sufficient to allow equilibrium to be attained, and in fact later work showed that above 60° C. equilibrium was attained within less than 2 minutes, so that the method might be adapted for amperometric titrations.

Effect of Diverse Ions. For ions causing precipitation of the dye the maximum tolerable amount was determined as that amount which failed to cause precipitation within a 2-hour period. Turbidity measurements were made with a photoelectric colorimeter using a 660 mμ broad-band filter. Results indicated that the introduction of sulfate, chloride, and potassium ions should be avoided. In amounts exceeding approximately 5 ml. per 50 ml., these ions cause the dye to precipitate. It is suggested that only perchloric acid or sodium hydroxide be used in any preliminary steps requiring an acid or base.
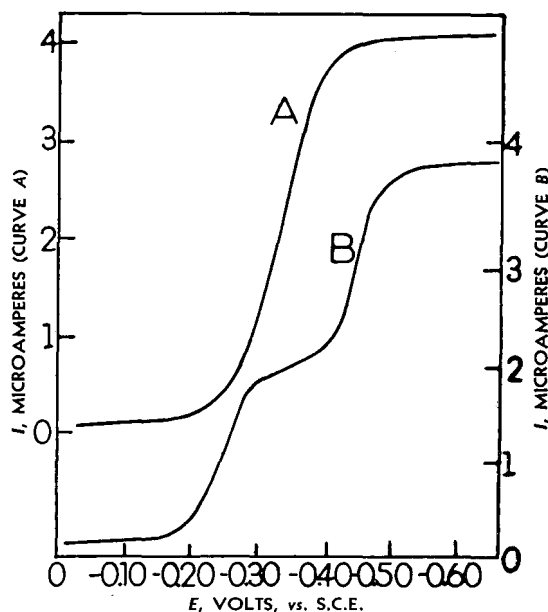

Figure 1. Current-Voltage Curves

A. Pontachrome Violet SW alone
B. Pontachrome Violet SW plus 9.28 micromoles of aluminum
Both solutions buffered at pH 4.7

To study the effect of other metals and various anions upon the aluminum-dye wave standard solutions were prepared from c.p. salts. These were tested by adding varying amounts to a series of solutions containing a fixed amount of aluminum and buffered at pH 4.7. Table II shows the results obtained in these tests. The noninterference of beryllium, even for concentrations exceeding one hundred times that of aluminum, is notable. Thus this procedure offers a new method for the determination of aluminum in the presence of beryllium. Large amounts of magnesium, calcium, zinc, and manganese are permissible. Titanium(IV) and vanadium(V) yield a second diffusion wave with the dye, but the wave is poorly defined although it seems

to be additive in the presence of aluminum. Fluoride forms a more stable complex with aluminum than does the dye; however, the presence of phosphate ions is permissible if not over ten times the aluminum concentration. The serious interference of iron, copper, nickel, and cobalt requires that these elements be removed completely. This can be accomplished by electrolysis at a mercury cathode (5).

NATURE OF ALUMINUM-VIOLET SW COMPLEX

To exhibit two distinct polarographic waves only in the presence of aluminum ions, the dye itself must exist in a different form when present in the complex. In view of Winkel and Siebert's work on azobenzene (10), it may be that the critical equilibrium involves the cis and trans forms of the dye. These authors observed two waves for azobenzene separated by 0.235 volt, although only the first wave was stable upon standing and it increased in height as the second wave decreased. Because a potential difference of 0.2 volt was found in the present work, and the first wave decreased in height as the aluminum-dye wave increased in height, it seems reasonable to believe that the aluminum ions might be stabilizing one of two tautomeric forms of the dihydroxyazo dye. Work is in progress in an attempt to elucidate the peculiar nature of the aluminum-dye complex.

Table II. Effect of Diverse Ions

Element Added	Element Added, Mg.	Aluminum Found, Mg.[a]
Arsenic(V)	10.0	0.098
Beryllium	10.0	0.098
Boron	10.0	0.103
Cadmium	5.0	0.105
	10.0	0.094
Calcium	100	0.103
	300	0.097
Copper	0.075	0.100
	0.150	0.103
	0.200	0.085
Cobalt	10.0	0.264
Fluoride	0.05	0.099
	0.10	0.085
Iron(III)	0.025	0.109
	0.050	0.119, 0.108
	0.100	0.121
Lead	2.0	0.250
Magnesium	10	0.100
	100	0.096
Manganese	10	0.103
	60	0.099
	120	0.081, 0.085
Molybdenum	0.25	0.093
Nickel	0.35	0.142
	8.0	0.245
Phosphorus(V)	1.0	0.102
	2.0	0.100
	3.0	0.040
Thorium	0.8	0.105
	4.0	0.124
Titanium	0.05	0.103
	0.15	0.130
	0.25	0.185
Vanadium(V)	0.120	0.200
	0.250	0.277
Zinc	10	0.100
	100	0.098
Zirconium	0.08	0.143
	0.8	0.183

[a] 0.100 mg. of aluminum present in each case.

DISCUSSION OF METHOD

The aluminum concentration present in the final polarographic solution should lie between 0.010 and 0.300 mg. of aluminum per 50 ml., although as little as 0.005 mg. may be detected. If the aluminum were present as a trace constituent, these amounts would correspond to a percentage range of 0.001 to 0.03% per

Table III. Analysis of Bureau of Standards Samples with Pontachrome Violet SW

Sample	Certified Value, % Al₂O₃	Value Found, %
Limestone 1a	4.16	3.92, 3.94
		4.36, 4.16
Dolomite 88	0.067	0.056, 0.056
Iron ore 26	1.03	1.12, 1.13, 1.18
		1.03, 1.03, 1.07
		1.17, 1.09, 1.06
		1.16, 1.16, 1.09
Magnetite ore 29	1.91	1.90, 1.77, 2.10
		1.71, 1.75
Silica brick 102	1.96	1.93, 2.04
		1.93, 1.90, 1.92
		2.00, 1.96
Soda-lime glass 128	1.89	1.61, 1.59
		2.14, 2.14
	% Al	
Manganese bronze 62	1.13	1.14, 1.14, 1.16
		1.18, 1.14, 1.14
62b	0.97	0.99, 0.99, 0.89
		0.94, 0.97
Phosphor bronze 63	0.05	0.045, 0.044, 0.054
		0.051, 0.046, 0.047
Zinc base alloy 94a	3.90	3.88, 3.97
		3.77, 3.67
		4.03, 3.96
		4.07, 4.07
Nitralloy steel 106	1.06	1.10, 1.11, 0.95
		1.08, 1.08
106a	1.07	1.12, 1.12, 1.08
		1.06, 1.10
High silicon steel 125	0.261	0.260, 0.270, 0.251
		0.260, 0.258, 0.269

gram of sample. It is not advisable to work with samples whose aluminum content exceeds 3 or 4%; otherwise the dilution factor becomes too large. Larger amounts of aluminum might be determined if the dye concentration were correspondingly increased, but then less inert salts could be present or else the dye would be precipitated.

The amount and nature of different ions present or added to the solution during the analytical manipulations preceding the polarographic step are very important. Only the perchlorate anion and acetate anion should be present in the sample or aliquot portion used to prepare the final polarographic solution. The total amount of perchlorate ion introduced must not exceed approximately 40 me. per 100 ml., including the amount neces-
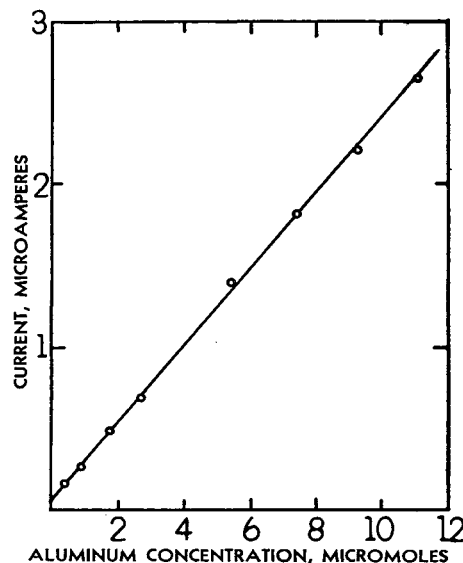

Figure 2. Relation between Diffusion Current and Concentration of Aluminum

sary to adjust the pH of the polarographic solution. This factor will limit the minimum amount of aluminum that can be handled. No appreciable amounts of potassium or ammonium ion should be present because of the slight solubility of their perchlorates.

The mercury cathode separation is the most suitable method for removing the heavy metals. The unitized style cell developed by Johnson and co-workers (4) and the procedures recommended by Parks and co-workers (5) give a clean, efficient separation. In siliceous materials, and even from the residual solution remaining after the mercury cathode electrolysis, aluminum can be separated from titanium, zirconium, and iron by fusion with sodium carbonate if these elements do not occur in more than about 100-fold excess over aluminum (6).

Results obtained for various Bureau of Standards samples are given in Table III.

PROCEDURE

Calibration Curve. To four 50-ml. volumetric flasks add enough standard aluminum solution to give 0.05, 0.1, 0.2, and 0.3 mg. of aluminum, respectively. Add a 10% sodium hydroxide solution until alkaline to methyl red, then a 5.0 N solution of perchloric acid until barely acid, and then an excess of 1.00 ml. of 5.0 N perchloric acid. Add 5.0 ml. of 2.0 N solution of sodium acetate and 20 ml. of 0.05% aqueous solution of Pontachrome Violet SW, and dilute to the mark. Immerse the flasks and contents in a beaker of water heated to between 55° and 70° C. for a 5-minute period, then cool the flasks to room temperature under tap water. Transfer the solution to the polarographic cell. Immerse the cell in a water bath maintained at 25.0° C. and bubble purified nitrogen through the solution for 15 minutes to remove dissolved oxygen. Run the polarograms between −0.1 and −0.8 volt versus the saturated calomel electrode and measure the diffusion currents of the second wave. Plot the wave height for each standard solution against the amounts of aluminum added. Draw the best straight line through the points (Figure 2).

For Steels. To determine acid-soluble aluminum, weigh samples containing 0.01 to 0.3 mg. of aluminum (or for larger amounts of aluminum weigh at least 0.1-gram samples) into a 50-ml. beaker and dissolve in the minimum amount of 5 N perchloric acid. Filter any insoluble residue through a loose-textured paper and wash with hot 1% perchloric acid. Transfer the filtrate to an electrolysis beaker. For 1.0-gram samples electrolyze for 20-, 20-, 20-, 30-, and 30-minute intervals at the mercury cathode with a current of 5 amperes. Change the mercury after each interval. For 0.1-gram samples three successive 20-minute electrolysis intervals are sufficient.

Evaporate the electrolyzed solution to 20 ml., and transfer the entire sample, or an aliquot, such that the final solution contains between 0.01 and 0.30 mg. of aluminum, to a 50-ml. volumetric flask. Treat the sample solutions in the same manner as the standards. Read the amount of aluminum from the calibration curve.

For Bronzes. Weigh samples containing 0.01 to 0.3 mg. of aluminum into a 50-ml. beaker and dissolve in 10 ml. of 5 N hydrochloric acid and 5 ml. of 30% hydrogen peroxide. After dissolution add 10 ml. of 5 N perchloric acid and evaporate to copious fumes of perchloric acid. Allow to cool, then transfer to an electrolysis beaker. Electrolyze for three 20-minute intervals at the mercury cathode with a current of 5 amperes. Change the mercury after each interval. Evaporate the electrolyzed solution to 20 ml. and transfer the entire sample, or a suitable aliquot, to a 50-ml. volumetric flask. Treat the sample solution in the
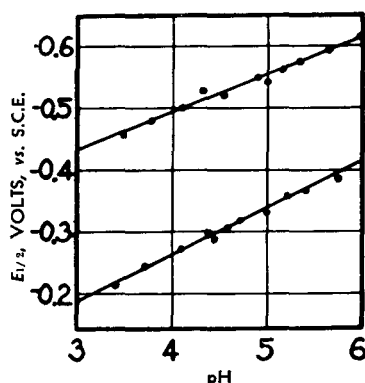

Figure 3. Relation of Half-Wave Potentials and pH

Upper. Aluminum-dye complex
Lower. Unreacted Violet SW

same manner as the standards. Read the amount of aluminum from the calibration curve.

For Minerals. For siliceous minerals and glasses (6), weigh a sample containing 0.02 to 0.6 mg. of aluminum oxide into a platinum crucible. Moisten with distilled water, and add 5 ml. of 48% hydrofluoric acid and 0.5 ml. of 36 N sulfuric acid. Heat to fumes of sulfuric acid. Cool, add 2 ml. of hydrofluoric acid, and evaporate to dryness. Ignite carefully until sulfuric acid ceases to be evolved. Add 2 grams of sodium carbonate and fuse for 10 minutes at the full temperature of a Meker burner. Dissolve the soluble portion of the melt with water, heat to boiling, and filter. Treat the entire sample, or an aliquot portion, in the same manner as the standards. Read the amount of aluminum from the calibration curve and convert to aluminum oxide. The factor is 1.89.

For siliceous minerals containing large amounts of heavy metals, alter the procedure as follows: Dissolve the residue from the hydrofluoric acid treatment in dilute perchloric acid and transfer to an electrolysis beaker. Electrolyze for suitable intervals at the mercury cathode (5), then treat the residual solution, or an aliquot portion, in the same manner as the standards.
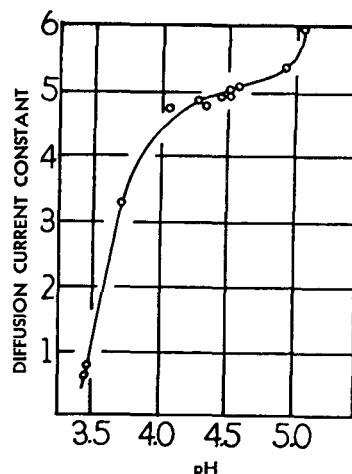

Figure 4. Diffusion Current Constant of Aluminum–Pontachrome Violet SW Compound as Function of pH

For other types of minerals, such as limestones, proceed with the ordinary scheme of analysis, and then fuse the R_2O_3 precipitate with sodium carbonate and proceed as described above.

LITERATURE CITED

(1) Ford, C. L., and LeMar, L., *A.S.T.M. Bull.* 157 (March 1949).
(2) Gull, H. C., *J. Soc. Chem. Ind.*, 56, 177 (1937).
(3) Heller, B. A., and Zan'ko, A. M., *Zavodskaya Lab.*, 8, 1030 (1939).
(4) Johnson, H. O., Weaver, J. R., and Lykken, L., ANAL. CHEM., 19, 481 (1947).
(5) Parks, T. D., Johnson, H. O., and Lykken, L., *Ibid.*, 20, 148 (1948).
(6) Parks, T. D., and Lykken, L., *Ibid.*, 20, 1102 (1948).
(7) Prajzler, J., *Collection Czechoslov. Chem. Commun.*, 3, 406 (1931).
(8) Volpi, A., *Gazz. chim. ital.*, 77, 473 (1947).
(9) Weissler, A., and White, C. E., IND. ENG. CHEM., ANAL. ED., 18, 530 (1946).
(10) Winkel, A., and Siebert, H., *Ber.*, 74B, 670 (1941).

Correction

N. Strafford has called our attention to the omission of a reference to the paper by N. Strafford and H. Crossley entitled "The Determination of Small Amounts of Sulphur in Certain Organic Compounds" [*Analyst*, 60, 163 (1935)] in the paper by R. E. Holeton and A. L. Linch entitled "Determination of Traces of Sulfur in Organic Compounds" [ANAL. CHEM., 22, 819 (1950)]. The reference was included in the original manuscript, but unintentionally omitted during one of the numerous revisions. The paper in question describes the determination of sulfur by a spray combustion method. We regret the oversight.

A. L. LINCH
R. E. HOLETON

Glycol Ethers as Nonaqueous Solvents in Polarographic Analysis

THOMAS D. PARKS[1] AND KENT A. HANSEN

Shell Development Company, Emeryville, Calif.

The ethylene glycol monoalkyl ethers (Cellosolves) are satisfactory nonaqueous solvents for the polarographic study of organic compounds. They are chemically and polarographically stable, dissolve certain polar materials to give conducting solutions, and are miscible with a wide variet y of materials including water and gasoline. Many nonpolar organic compounds as well as metal ions give satisfactory polarograms in these nonaqueous media.

THE polarographic study of nonpolar organic compounds, or of impurities in such compounds, often requires the use of a completely nonaqueous solvent in order to achieve solution of the sample. The number of useful solvents is limited because they must be able to dissolve a polar salt to act as a supporting electrolyte. Another requirement is that the solvent itself be not easily reduced at the dropping mercury electrode. For polarographic studies of organic compounds, Kolthoff and Lingane (3) report the use of water with alcohols, polyhydric alcohols, and glacial acetic acid. Laitinen, Wawzonek, and coworkers (4, 6, 7) describe the use of solutions composed of dioxane and water for the polarographic study of a wide variety of organic compounds.

Of a large number of organic solvents tested in this investigation, the ethylene glycol monoalkyl ethers (Cellosolves) were found to fill most completely the requirements listed above. These materials are miscible with water or gasoline and will dissolve such salts as the tetraethylammonium halides to form conducting solutions. They are not easily reduced at the dropping mercury electrode and their solutions are stable, neither decomposing nor forming high concentrations of peroxides when kept in stoppered bottles.

EXPERIMENTAL

Difficulties were experienced with the use of the dioxane-water medium, proposed by Laitinen and Wawzonek (4) and adopted by Burdett and Gordon (1), in the determination of naphthalenes in kerosene or light gas oil. Peroxides formed rapidly in the dioxane upon standing and gave interfering reduction waves which preceded those of naphthalene. Although satisfactory results were obtained when freshly purified dioxane was used in the preparation of the electrolyte, the sensitivity of the method was often impaired because of the limited miscibility of the hydrocarbon sample in the mixture. For the same reason, the dioxane-water medium was also found to be inadequate for the direct determination of tetraethyllead in gasoline (2).

Ethylene chloride, ethylene bromide, benzene-methanol mixtures, dioxane, methyl Carbitol (diethylene glycol monomethyl ether), Carbitol (diethylene glycol monoethyl ether), methyl Cellosolve (ethylene glycol monomethyl ether), and Cellosolve (ethylene glycol monoethyl ether) were tested for use as solvents in polarographic analyses using purified tetra-n-butylammonium iodide (Eastman Kodak reagent, recrystallized twice from ethyl acetate) as the supporting electrolyte. Petroleum samples were not soluble in the alcohols and Carbitols. Ethylene chloride gave a reduction wave at approximately -1.8 volts and ethylene bromide at approximately -1.4 volts *vs.* the saturated calomel

electrode at 25° C. Freshly prepared 1,4-dioxane was found generally satisfactory; the main objection to its use was the difficulty of purification and rapid deterioration in storage.
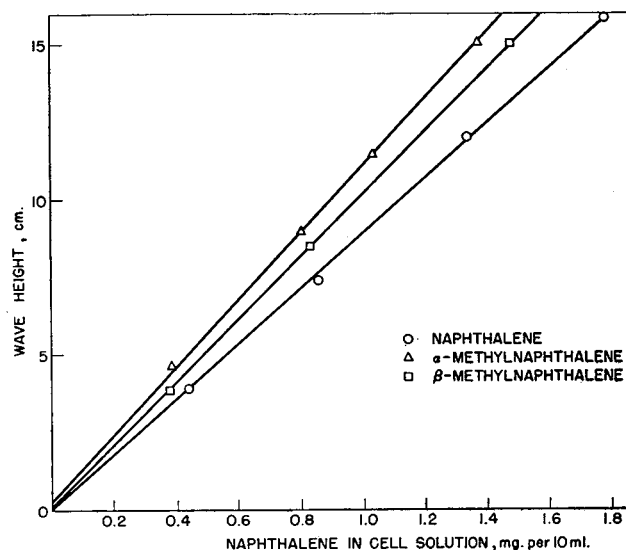

Figure 1. Polarographic Calibration Curves for Naphthalene in Methyl Cellosolve

Electrolyte, 0.1 N tetra-n-butylammonium iodide
Sensitivity, 0.0383 ma. per mm.
Temperature, 25° C.

The purification of the Cellosolves was found to be relatively simple. About 2 liters of the solvent were refluxed in a round-bottomed distillation flask for 30 minutes with 20 grams of anhydrous ferrous sulfate and distilled using a simple 3-plate column. After 6 months' storage, the solvents thus purified did not show any reducible impurities. Oxygen may be conveniently removed from Cellosolve electrolytes by purging with nitrogen, but for approximately three times longer than is ordinarily required for aqueous electrolytes. For the naphthalene determination, a solution of 0.1 N tetra-n-butylammonium iodide in methyl Cellosolve was found to be a satisfactory electrolyte. Typical calibration curves obtained for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in this electrolyte are shown in Figure 1. The use of this Cellosolve electrolyte greatly extended the useful range of the naphthalene calibration curve because naphthalene is more soluble in it than in the dioxane electrolyte containing 20% of water, which can dissolve only approximately 0.3 mg. naphthalene per milliliter.

An application of a Cellosolve solvent was found in the direct polarographic determination of tetraethyllead in gasoline (*2*). The gasoline samples were dissolved in Cellosolve containing a sufficient concentration of hydrogen chloride to decompose the tetraethyllead; after heating at steam temperature, the resultant solution was electrolyzed at the dropping mercury electrode for lead content. As the half-wave potential for lead ion is 0.4 volt (*vs.* the mercury pool anode), it was not necessary to purify the Cellosolve (Figure 2). The success of this determination suggests the possible analysis of other metallo-organic compounds in gasoline or similar solvents.
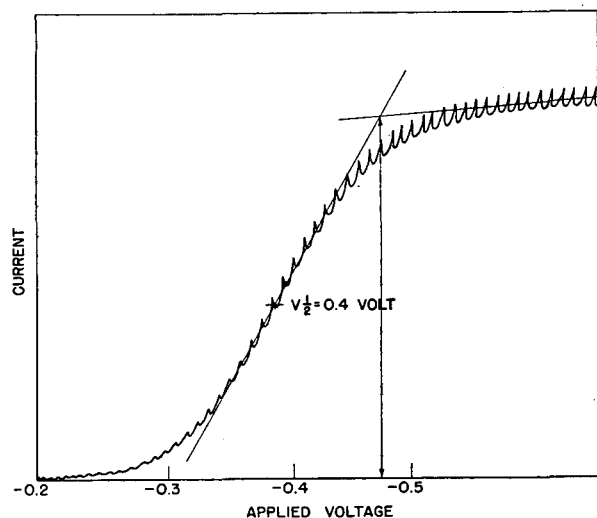
Figure 2.   Typical Lead Polarogram

After decomposition of tetraethyllead in 1 *N* hydrogen chloride in Cellosolve

No maxima were observed in the application of Cellosolve electrolytes to either the tetraethyllead or the naphthalene determinations. However, maxima have been encountered in lead reduction waves where the lead was added to the Cellosolve–hydrogen chloride electrolyte as lead acetate; in this case, methyl red was found to be an efficient suppressor. The dif-

fusion current of lead in Cellosolve–hydrogen chloride electrolyte was found to be approximately one eighth of that obtained from the same concentration of lead in an aqueous hydrochloric acid electrolyte.

Müller has pointed out that the necessity for using well buffered solutions for the analysis of reducible organic compounds (*5*) and the change of half-wave potentials with a change in hydrogen ion concentration. Well buffered solutions were obtained in Cellosolve using acetic anhydride–sodium acetate and ammonium hydroxide–tetraethylammonium bromide mixtures.

DISCUSSION

A brief study of the use of the glycol ethers as solvents for polarographic analysis showed they have promise in the analysis of organic compounds. Cellosolve dissolves a wide variety of polar and nonpolar organic compounds, organometallic compounds, inorganic acids, and salts. It generally allows a choice of supporting electrolyte and dissolves sufficient salts for buffer systems. While the present investigation of organic compounds in the Cellosolve medium was confined to naphthalene, 1- and 2-methylnaphthalene, and tetraethyllead, it is believed that the Cellosolves make possible the polarographic study of a wide variety of compounds.

ACKNOWLEDGMENT

The authors wish to thank C. W. Smith for his suggestion of the use of the glycol ethers for this purpose.

LITERATURE CITED

(1) Burdett, R. A., and Gordon, B. E., Anal. Chem., **19**, 843–6 (1947).
(2) Hansen, K. A., Parks, T. D., and Lykken, Louis, *Ibid.*, **22**, 1232 (1950).
(3) Kolthoff, I. M., and Lingane, J. J., "Polarography," p. 344, New York, Interscience Publishers, 1941.
(4) Laitinen, H. A., and Wawzonek, S., *J. Am. Chem. Soc.*, **64**, 1765–8 (1942).
(5) Müller, O. H., *Chem. Rev.*, **24**, 95–124 (1939).
(6) Wawzonek, S., and Laitinen, H. A., *J. Am. Chem. Soc.*, **64**, 2365–8 (1942).
(7) Wawzonek, S., Laitinen, H. A., and Kwiatkowski, S. J., *Ibid.*, **66**, 830–3 (1944).

Manual Polarograph for Rapid Determinations of Lead and Cadmium in Zinc

L. C. COPELAND AND F. S. GRIFFITH, *The New Jersey Zinc Company (of Pa.), Palmerton, Pa.*

THE spectroscope has been generally used by The New Jersey Zinc Company (of Pa.) for rapid routine determinations of cadmium and sometimes of lead. Lead was most frequently determined by electrolysis. The desirability of providing better analytical service for one plant, for which no spectroscope was available, prompted this investigation of the use of the polarograph.

The literature contains numerous references—many of which are given by Kolthoff and Lingane—to the successful application of the polarographic method to this analysis. The more recent work of Nickelson and Randles (*5*), Hawkings and Thode (*2*), and Ford (*1*) among others present thorough studies of this method. This paper describes a simplified modification of this method, particularly the use of a relatively inexpensive manually operated

polarograph and spot readings at specific voltages for rapid routine determinations. In this laboratory the new method, in the hands of operators of limited experience, has successfully replaced spectroscopic and electrolytic determination of impurities in zinc.

Figure 1 shows a typical polarographic curve for the determination of lead and cadmium in acid zinc chloride solutions as obtained by conventional procedures using a recording polarograph. The abscissa indicates the voltage applied between the dropping mercury cathode and the mercury pool anode and the ordinates show the diffusion current obtained at each potential.

The "wave height" is proportional to the concentration of the ion being reduced, and in this example the solution being analyzed contained 0.237% lead and 0.071% cadmium. The usual pre-

An inexpensive manual polarograph is described with which it is possible to determine lead and cadmium in zinc rapidly by obtaining the diffusion currents at only three potentials. Inexperienced personnel can easily handle four samples an hour, including solution of the metal, with an accuracy comparable to the more conventional spectroscopic and chemical methods. Other applications of this machine and method are mentioned.

cautions were followed of degassing the solution and maintaining the same temperature, drop rate, and supporting electrolyte as in the calibration of the capillary. No interfering ions were encountered in the determinations on high purity zinc samples but in some of the cases cited, procedures were developed to eliminate interference from tin and indium.

PROCEDURE

Twenty grams of zinc were weighed into a 600-ml. beaker and 10 to 15 ml. of water were added, followed by 70 ml. of hydrochloric acid (specific gravity 1.19). As the initial vigorous reaction subsided, two drops of a 3% cobalt chloride solution were added as catalyst. The solution was completed on the hot plate (undissolved copper may be left as a washed decant residue with no detectable loss in lead or cadmium), cooled, and diluted to 100-ml. volume.

THREE-POINT DETERMINATION

Lingane (4) and others have suggested that, if sufficiently routine procedures are developed, it is only necessary to measure the difference in the residual current between fixed potentials on each side of the appropriate "half-wave potential" to determine the concentration of a given ion. Figure 1 also illustrates the application of this method to the lead and cadmium waves in zinc. Here it is obvious that the vertical distance between A and B and B and C is proportional to the wave heights of lead and cadmium, provided that:

1. A correction is made for the vertical component of the charging current—that is, by subtracting $a + b$ from the vertical distance, AB, in Figure 1. In general, this correction was needed only when using shunts with multiplication factors less than 10.
2. The position of A, B, and C is definitely fixed in the straight-line portion of the residual current.
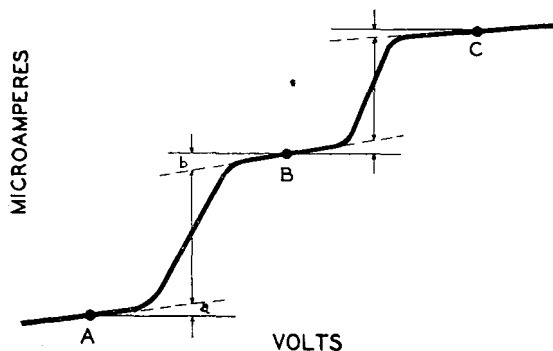

Figure 1. Polarographic Curve

Lead and cadmium in acid zinc chloride solution

The photographic machine requires 6 minutes to run through the 0.5-volt range, a little time to develop the paper, and finally about 3.5 minutes to measure the heights of the two waves. In contrast, the measurement of the diffusion currents at the three potentials can be completed in 3.5 minutes. The saving in time prompted a comparison of the accuracy of the two methods.

The voltages chosen to test the method were 0.30, 0.50, and 0.70 volt against a silver–silver chloride anode (lead half-wave at 0.42 volt, cadmium half-wave at 0.59). Measurements were made at the three voltages mentioned on the photographic records previously obtained in the preliminary investigation, and the results were calculated and compared with the results obtained by the more usual procedure. Excellent agreement was obtained by the two methods. In addition, the three-point method was used for four to ten daily samples for a 25-day period and the results were compared with the spectroscopic results on cadmium and the electrolytic results on lead. These samples ranged from 0.0002 to 0.0017% lead and from 0.0001 to 0.0043% cadmium. Over two hundred such comparisons were made with no significant discrepancy appearing.

INEXPENSIVE MACHINE FOR ROUTINE WORK

Because of the apparent reliability and speed of this simplified procedure, an inexpensive machine was assembled to free the recording machine for other exploratory work and special analysis
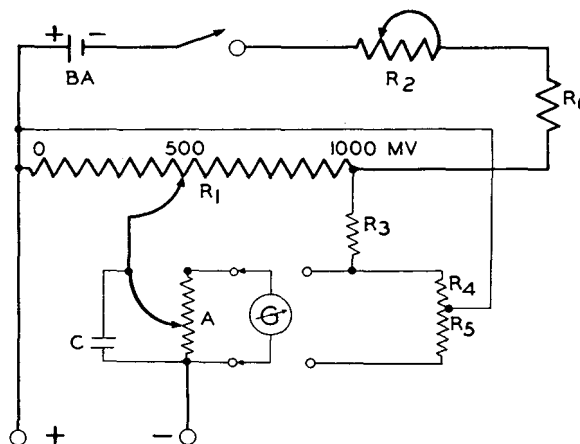

Figure 2. Wiring Diagram of Manual Polarograph

Figure 2 shows the wiring diagram for the polarograph which was assembled from standard equipment; this is similar to the simple circuit given by Kolthoff and Lingane (3).

The galvanometer, G, serves a dual purpose, acting as an accurate voltmeter in the standardization circuit and as a microammeter in the polarographic circuit. The potentiometer, R_1, is a ten-turn helical coil with a total resistance of 10 ohms. The knob of this unit is graduated from 0 to 100, and a second concentric dial indicates the turn; consequently, with a potential of 1 volt established across the resistance, a direct-reading slide wire graduated in divisions of 1 mv. results.

Figure 3 is a photograph of this machine. Similar manual machines are commercially available.

Routine procedures have been facilitated by a cell design permitting shifting of the mercury capillary and silver reference electrodes to the various thermostated and outgassed solutions.

Table I. Standard Sample of Zinc Submitted as Unknown

No. detns.	Lead, % 263	Cadmium, % 263
Average value	0.00142	0.000897
σ	0.000072	0.000053
Error made by 1-mm. mistake in galvanometer reading	0.00011	0.000062
Previously accepted value	0.0014	0.0009
Method	Electrolysis	Sepn. as CdS, electrolysis

The cells consisted of the lower half of test tubes with the rim flared so as to support the tubes in holes cut in a thick aluminum plate which covered the constant temperature bath. The inert gas was passed in series through a group of the cells, each cell having a rubber stopper carrying inlet and outlet tubes. At the start of the determination, the degassing head of a cell was replaced by a stopper carrying gas inlet and outlet, the dropping mercury capillary, and a silver chloride reference electrode. The silver anode is in the form of a helix passing around the gas inlet tube and capillary; it terminates above the end of the capillary in order to avoid amalgamation of the silver. After transferring the cell heads the cell is degassed for an additional 0.5 minute before taking the readings.

As a check on the accuracy of this method of analysis, a standard sample of high purity zinc was submitted frequently as an unknown over a period of 8.5 months. Four different operators working at different times totaled 263 separate solutions of the zinc and determined both cadmium and lead. Table I shows the results of this study and Table II shows the distribution of the polarographic results. The accuracy of this polarographic method is very high.

Table II. Distribution of Polarographic Results

(Standard zinc as unknown; four analysts)

| Lead		Cadmium	
Value, %	No.	Value, %	No.
0.0012	5	0.0006	1
0.0013	17	0.0007	4
0.0014	165	0.0008	26
0.0015	68	0.0009	205
0.0016	6	0.0010	26
0.0017	1	0.0011	1
0.0018	1		263
	263		

This method has made possible considerable saving in man-hours, elapsed time, and chemicals. Where previously 5 hours were required to determine lead and 3 hours to determine cadmium on four samples per shift of high purity zinc, results are now reported within 1 hour. The saving in elapsed time is often much greater; samples received at 3:30 P.M. are now reported at 4:30 P.M. whereas previously they were not reported until 8:00 A.M. the following morning. A two-man night shift formerly occupied mainly with these determinations has been eliminated. In general an experienced man requires approximately 30 minutes for the first sample and about 6 minutes for each additional sample to determine both lead and cadmium. Although iron may be determined polarographically in the presence of organic acids and carefully controlled pH, the colorimetric thiocyanate method is more satisfactory.

INTERFERING ELEMENTS

When analyzing impure metal samples containing tin and indium, the results for lead and cadmium are high. Since tin is completely precipitated above pH 3 and indium above pH 5.5, whereas lead and cadmium do not precipitate below pH 6 when present in moderate amounts, the tin and indium may be removed by adjusting the pH of the solution with ammonia to a pH of 5.6 at room temperature. Good agreement with electrolytic deposi-

tion has been obtained by this method on samples known to contain tin and indium and analyzing as high as 0.82% lead and 0.18% cadmium. Some success has also been obtained in determining tin by the difference between the lead waves in acid solutions after pH adjustment. It appears likely that indium can be determined from the cadmium wave in a similar manner. As mentioned previously, copper may be filtered off after solution of the sample in hydrochloric and boiling for a few minutes. Generally, some copper is dissolved but not enough to cause trouble. No appreciable loss has been found in lead or cadmium.

Many other uses are being found for this machine such as the determination of zinc in soils. This method is superior to the dithiozone method in sensitivity and accuracy although it is not as readily applicable to field work. Recently 24 soil samples were analyzed for zinc using the manual polarograph and two-point readings. The zinc content varied from 0.02 to 0.4%. After solution and dehydration of the samples (1 gram), less than half a day was required by one man to obtain the results. This work would have required about a week if determined by the dithiozone method.
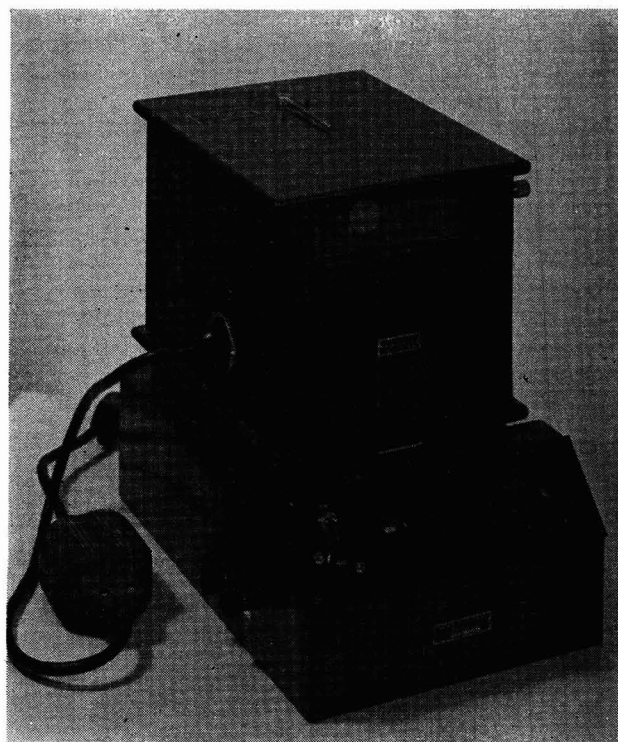
Figure 3. Manual Polarograph

As a further example of the reliability of the system a second instrument was constructed and shipped to another plant where analysts with no previous polarographic experience quickly mastered the technique on the basis of a few typewritten instructions.

LITERATURE CITED

(1) Ford, E. G., *Can. Chem. Process Inds.*, **33**, 1051 (1949).
(2) Hawkings, R. C., and Thode, H. G., IND. ENG. CHEM., ANAL. ED., **16**, 71 (1944).
(3) Kolthoff, I. M., and Lingane, J. L., "Polarography," New York, Interscience Publishers, 1941.
(4) Lingane, J. L., private communication.
(5) Nickelson and Randles, "Polarographic and Spectrographic Analysis of High Purity Zinc and Zinc Alloys for Die Casting," London, H.M. Stationery Office, 1945.

Use of High Frequency Titrimeter

Volumetric Determination of Beryllium

KERMIT ANDERSON AND DAVID REVINSON[1]
Fairchild Engine and Airplane Corporation, Oak Ridge, Tenn.

A new high frequency titrimeter using a grid dip–type oscillator was investigated as an instrumental method for assay of beryllium metal and for determination of beryllium in beryllium oxide and beryllium carbide. The instrument proved to be adaptable to the titration of beryllium ion with sodium hydroxide. The titration of beryllium ion furnishes a new example in which the high frequency titrimeter has served to indicate the end point of a volumetric reaction involving an amphoteric substance.

BERYLLIUM has been one of the few remaining elements that has successfully withstood an accurate analysis by volumetric means (*2, 5, 6*). Since a suitable method for the rapid volumetric assay of beryllium metal was needed in these laboratories, it was decided to investigate the use of the grid-dip oscillator-type titrimeter (*3*) for this purpose.

Jensen and Parrack (*4*) and West (*7*) have adequately reviewed the literature on the rapid development of this instrument. Since the great majority of modern developments in beryllium chemistry are under security restriction, it is somewhat difficult to give an adequate review of the analytical chemistry of this element.

With the electronic titrimeter, reasonably satisfactory results could be obtained using either triammonium phosphate, ammo-

nium hydroxide, or sodium hydroxide as the titrating agent However, sodium hydroxide gave the sharpest breaks in the titration curve and hence was selected as the most promising for this work. Work is expected to continue with various reagents for volumetric analysis of beryllium in these laboratories.

APPARATUS AND REAGENTS

The titrimeter used in this work was a grid-dip oscillator-type instrument developed and built in the NEPA laboratories. The instrument and its operating characteristics are more fully described in an earlier paper by Anderson, Bettis, and Revinson (*1*).

Standard 0.5 *N* sodium hydroxide was used. It was kept in wax-lined bottles, free from carbon dioxide. Standard beryllium solution was prepared by dissolving National Bureau of Standards beryllium metal No. 2700 in a slight excess of 1:1 hydrochloric acid and then evaporating to near dryness. Distilled water was
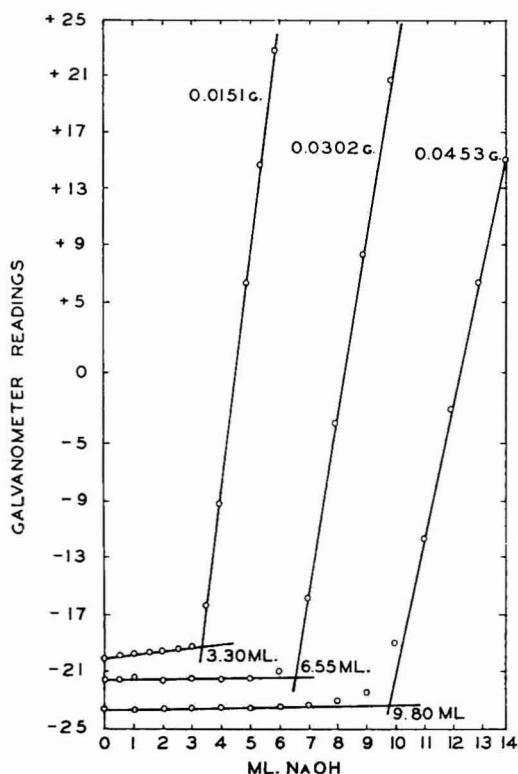
Figure 1. Titration of Beryllium with Sodium Hydroxide

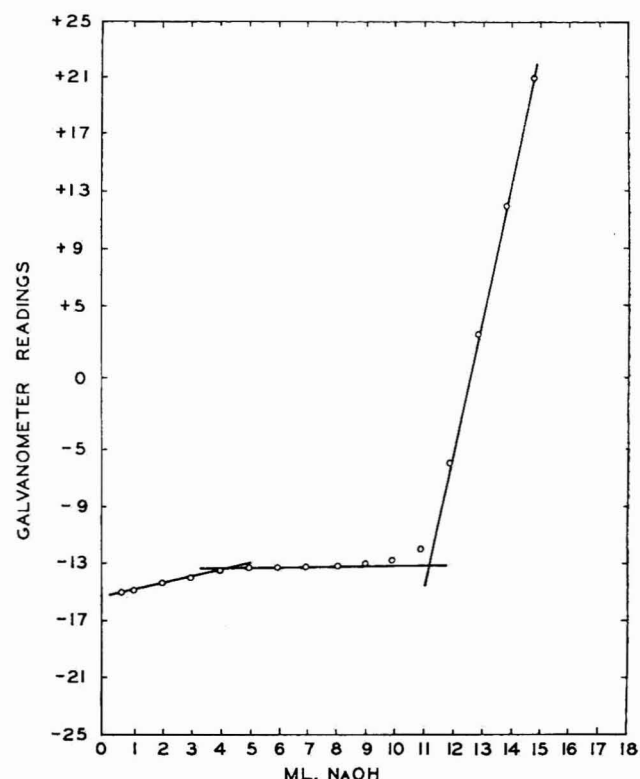100 ml. of solution titrated with 0.4320 *N* NaOH in air at 22 mc.

Figure 2. Beryllium Titration in Air in Presence of Free Hydrochloric Acid

0.0302 gram of Be in 100 ml. of solution containing free HCl titrated with 0.4320 *N* NaOH in air at 22 mc.

added and evaporation was repeated. After adding more water and filtering if necessary, the solution was diluted to a known volume, the pH of which was about 5.2. This procedure was followed in order to remove the free hydrochloric acid that was present. An aliquot was taken and a precipitation carried out using ammonium hydroxide to gravimetrically determine the amount of beryllium present.

PROCEDURE

Preliminary work with the titration showed that the entire system had to be kept free of carbon dioxide. The analytical procedure was carried out in hydrochloric acid solution to eliminate all chance of dissolving carbon dioxide, and the titration was carried out under an inert atmosphere of nitrogen or argon.
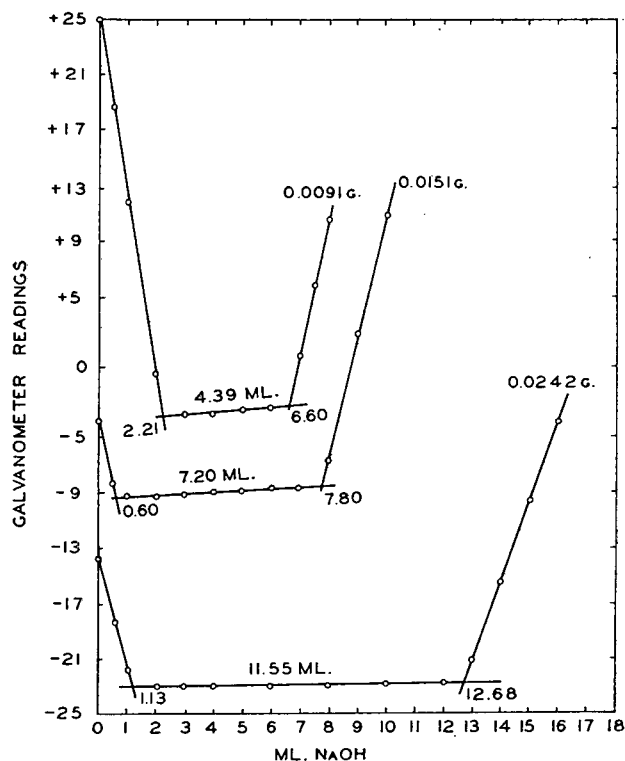
Figure 3. Beryllium and Free Hydrochloric Acid Titrated with Sodium Hydroxide

100 ml. of solution containing indicated weight of Be titrated with 0.4320 N NaOH at 22 mc. using carbon dioxide-free reagents in nitrogen atmosphere

Owing to the presence of hydrochloric acid in the solution to be titrated, two breaks in the titration curve were observed, the first for the free acid and the second corresponding to the end point for the beryllium titration. Care was taken to ensure that a reasonable amount of free acid was present in the solution, and a calibration curve was set up plotting the quantity of sodium hydroxide used between the two breaks in the titration curve against quantity of beryllium present.

The solution to be titrated was placed within the condenser plates in a 37-mm. test tube. After allowing the titrimeter 10 minutes to warm up, the range was first set to obtain, over the whole galvanometer scale, the total current change given during the complete titration. This was obtained by preliminary runs and was satisfactory for other similar samples. Once the range was determined, the current from the battery was adjusted to balance the grid current at whatever portion of the galvanometer scale the readings were to be started. Upon titrating in the normal manner and plotting grid current against volume of reagent added, the end points could be detected by sharp breaks in the curve.

Of the interchangeable coils available to give the necessary high

frequencies, 22 mc. was arbitrarily chosen as it appeared that one frequency did not have any advantage over another; for other analyses this may not be true. The following was the analytical procedure used:

1. The sample was accurately weighed, dissolved in 6 N hydrochloric acid, and evaporated to near dryness; it was left on a steam bath until a cake formed.
2. The sample was then made up to 100 ml., and aliquots containing not more than 40 mg. of beryllium were pipetted into a 37-mm. test tube.
3. Five milliliters of 0.5 N hydrochloric acid were added, and the sample was gently boiled to free from carbon dioxide, stoppered, and cooled under water.
4. The stopper was removed and the tube was filled with nitrogen gas (any other inert gas may be used), placed in the titrimeter, and diluted to 100 ml. with carbon dioxide-free water. The solution was titrated while passing nitrogen over the surface.
5. The results were plotted, and the required volume of sodium hydroxide and weight of beryllium were determined from the curves.

The 5 ml. of 0.5 N hydrochloric acid was used to make sure that the first break was not overlooked. Experience has shown that gentle boiling for a short time gives anywhere from 0.5 to 3 ml. for the first break. This must be found, or the titration will be in error. Starting with the addition of 0.5 N hydrochloric acid, and using a suitable beryllium aliquot, this procedure was also used in obtaining data for the calibration curve.

RESULTS

Figure 1 shows the curves obtained when different amounts of beryllium were present and the titration was carried out in air. A definite relationship was observed between the amount of beryllium present and the amount of titrant required. However, when acid was present, as must be the case in routine sample analysis, the curve in Figure 2 was obtained. The flatness of the acid neutralization curve invalidates this type titration. In order to overcome this difficulty, the titrations were performed using carbon
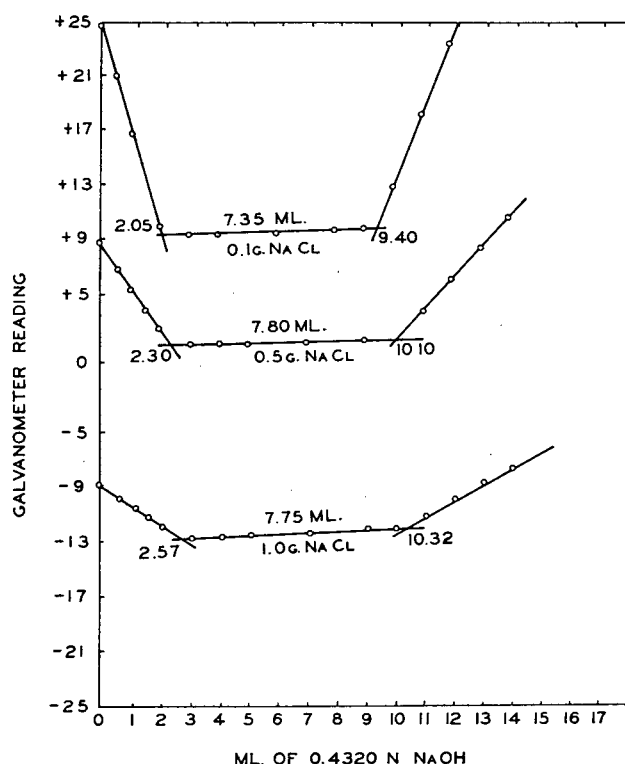

ML. OF 0.4320 N NaOH
Figure 4. Effect of Sodium Chloride

0.0151 gram of Be in 100 ml. of solution titrated with carbon dioxide-free reagents in nitrogen atmosphere at 22 mc.

dioxide–free reagents and inert gas as the covering atmosphere. Figure 3 shows the type curve obtained when varying amounts of beryllium and acid were present. By subtracting the initial end point from the final end point, the volume of sodium hydroxide used in the actual titration could be determined. This volume is proportional to the amount of beryllium present.

Table I. Results of Several Analyses

Be Present, Mg.	Be Found, Mg.	Difference, Mg.
12.7	12.8	0.1
15.9	15.7	−0.2
27.5	27.5	−0.0
37.1	37.3	0.2

For the same amount of beryllium, a titration carried out in air gave about one half the amount of titrating agent required when the same titration was carried out in an inert atmosphere.

Other metals that form insoluble hydroxides will give erroneous results. If an excess of sodium chloride was present, the curves were affected as shown in Figure 4. The larger the amount of salt present, the smaller the slope became and with it, a deviation from the amount called for in the calibration curve appeared. The deviation was such that the titrant tended to approach the stoichiometrical amount. This is desirable, but reproducibility was not obtainable and, consequently, it seemed better to titrate with as little sodium chloride present as possible.

As a check on the accuracy of the method, the data in Table I are presented. The analyses were run on carefully prepared known samples.

CONCLUSIONS

The use of high frequency oscillators has made possible the analyses of beryllium by accurate volumetric means. Since other metals and large amounts of salts may tend to obscure end points, care must be taken to prepare the sample so that optimum conditions for the titration are attained.

LITERATURE CITED

(1) Anderson, Kermit, Bettis, E. S., and Revinson, D., ANAL. CHEM., 22, 743 (1950).
(2) Evans, B. S., *Analyst*, 60, 291–3 (1935).
(3) Furman, N. H., "Scott's Standard Methods of Chemical Analysis," 5th ed., pp. 139–43, New York, D. Van Nostrand Co., 1940.
(4) Jensen, F. W., and Parrack, A. L., IND. ENG. CHEM., ANAL. ED., 18, 595–9 (1946).
(5) Tarayan, V. M., *Zavodskaya Lab.*, 12, 543–6 (1946).
(6) Torley, R., and Waldbauer, L., *Proc. Iowa Acad. Sci.*, 48, 277 (1941).
(7) West, P. W., Burkhalter, T. S., and Broussard, L., ANAL. CHEM., 22, 469 (1950).

Amperometric Titration of Fluoride with Lead

HENRY G. PETROW AND LEONARD K. NASH

Harvard University, Cambridge, Mass.

The amperometric titration of fluoride ion with lead, in a solution of 0.1 *M* chloride ion, is described. Lead chlorofluoride precipitates smoothly if the pH of the solution falls in the range 5.5 to 6.5 and if the ionic strength of the solution is not excessive. The difficult separation of this fairly soluble precipitate from its mother liquor is completely avoided. Simple equipment is sufficient, thermostating is unnecessary, the solutions need not be deaerated, small concentrations of anions other than sulfate do not interfere, and the complete determination takes less than 1 hour. Under favorable conditions the results are accurate to a few tenths of a per cent.

IN A recent publication (*4*) Kaufman has called attention to some weaknesses in the determination of fluoride ion by a widely practiced volumetric procedure. This method (*2*) involves the precipitation of the fluoride as lead chlorofluoride, followed by a separation of the precipitate from the mother liquor and a Volhard titration of the chloride content of the precipitate. A difficult quantitative separation of a relatively soluble—0.32 gram per liter of water at 25° C. (*2*)—precipitate from its mother liquor is involved, and considerable experimental manipulation, including two quantitative filtrations, is required. The method involves the addition of large excesses of solid reagents, and the original precipitate must age overnight. Kaufman indicates the necessity for particularly precise pH control between the narrow limits of 4.60 and 4.70, but even then it is not possible to secure results of an accuracy surpassing 1%, or to work with quantities of fluoride less than about 15 mg.

There is also an indirect lead chlorofluoride method (*7*) in which this salt is precipitated by the addition of excess lead and a measured excess of a determinate chloride solution to a chloride-free solution of the unknown fluoride. After filtration of the precipi-

tate the excess chloride is determined by argentometric titration of an aliquot of the filtrate. This procedure also requires that the primary precipitate be aged for 12 hours; also, quantities of fluoride less than 19 mg. have not been determined.

The distinctive advantages of the clean lead chlorofluoride precipitation can be retained in a swifter and more generally satisfactory procedure based on the direct amperometric titration of fluoride with lead in a chloride medium. In that it eliminates the necessity of making a quantitative precipitation and/or a quantitative separation of the precipitate, the amperometric, like the conductometric, determination of the end point is particularly valuable when, as in the authors' determination, the precipitate is appreciably soluble. Langer reports (*6*) that in dilute fluoride solutions this titration fails because of the slowness with which the precipitate separates. However, after the authors initiated this investigation, Haul and Griess (*1*), working with higher concentrations of fluoride, described both polarographic and amperometric titration procedures based on the precipitation of lead chlorofluoride.

In the polarographic procedure a determinate chloride-bearing

solution of lead nitrate is twice examined polarographically to determine the height of the lead wave before and after the addition of an aliquot of the fluoride solution. The apparent diminution of the lead concentration, after correction for dilution and for the solubility of the lead chlorofluoride, reflects the quantity of the added fluoride. This procedure stresses precise polarography, particularly when small quantities of fluoride are measured in terms of the differences between much larger amounts of lead. Also, if the fluoride solution contains substances which undergo polarographic reduction at about the same potential as lead, the simple determination is vitiated. Furthermore, the correction for the dissolved lead chlorofluoride, which must be separately determined with the aid of the mass action law, is not a well-defined constant but a complex variable of the composition of the solution and the temperature. The combined temperature dependence of the solubility correction and the polarographic wave height introduce a substantial temperature coefficient of 3.4% per ° C., and thermostating is essential. This technique presents no obvious advantages.

The amperometric titration procedure of Haul and Griess is more conventional; it involves the portionwise addition of a determinate lead nitrate solution to a chloride-bearing solution of the unknown fluoride. This titration is carried out in 50% alcohol and is said to determine fluoride concentrations in the range 1 to 50 millimolar with an average accuracy of about 1%. However, no experimental results are cited in support of this claim, nor is the pH prevailing in these experiments stated. The end points were defined by that volume of the lead nitrate titrant at which the observed diffusion current equals a value, established in trials with knowns, which corresponds to the stoichiometric point. Such a method fails with abnormally large fluoride samples when the composition of the solution—for example, its alcohol content—deviates from the composition prevailing in the trials made with knowns. Under such circumstances several trials should be made with known amounts of fluoride, and the apparent end points should be determined from the intersections of the linear arms of the titration curves. A standardization curve is then constructed by plotting the titrant volumes corresponding to the apparent end points against the coordinated quantities of fluoride; this line is then used in interpreting the results secured with unknown amounts of fluoride. Such a tedious standardization suggests that the stoichiometric point does not coincide with the apparent end point in this titration, but the extent of the deviation is not indicated.

The authors' investigations indicate that the amperometric titration procedure can be made simpler and more precise by a better definition of experimental conditions. Under these conditions the extrapolated end point and the stoichiometric point coincide within the experimental error—a few tenths of a per cent.

APPARATUS AND PROCEDURE

A manual polarograph (5) with dropping mercury electrode was employed. The applied potential was read with reference to a saturated calomel electrode using a Type K-2 potentiometer; the polarographic currents were measured with the same instrument in terms of the potential drops produced in a 10,000-ohm resistance in series with the cell. The sensitivity of the indicating galvanometer was controlled with a variable shunt. The titration cell was a small beaker, internally coated with paraffin; into this beaker dipped a salt bridge connected with the saturated calomel electrode.

The measurements were all made with the dropping electrode at a potential of −1.0 volt against the saturated calomel electrode. A few trials made with this potential set at −0.8 volt did not disclose any noticeable differences in behavior. Although oxygen is reduced at these potentials, preliminary investigation indicated that deaeration of the solutions did not materially affect the analytical results. Consequently this time-consuming operation was abandoned and all but one of the solutions were used in an air-saturated condition.

A weighed sample of sodium fluoride was added to the beaker and dissolved in about 100 ml. of 0.1 M potassium (or sodium) chloride. After adjusting the pH with dilute hydrochloric acid,

the titrant—0.1 M lead nitrate—was delivered to the beaker from a calibrated 10-ml. buret. The solution was stirred manually, and the current readings were taken from 3 to 5 minutes after each addition of titrant. In a few cases in which the current was measured for periods of up to 1 hour the drift of the amperometric readings, after the first few minutes, was only 1 to 2%. The average time required for the complete titration did not exceed 1 hour. All measurements were made at room temperature without special thermostatic control. The results of a number of trials are shown in Table I.

DISCUSSION OF RESULTS

The acidity of the titration solution was of pivotal importance, although great delicacy in its control was unnecessary. When the initial pH of the fluoride solution was 5.2 or less, the amperometric readings were ill-defined and subject to large drifts; they were also inadequate for defining the end point satisfactorily. Good results were secured with an initial pH of 5.5 or 6.5. One successful trial was made at an initial pH of 7.0, but the readings here and at higher pH's were not as satisfactory. Thus, results of optimum accuracy are secured only when the initial pH is not less than 5.5 and not greater than about 6.5. Typical curves secured under these conditions are shown in Figure 1. The titration curve obtained at the higher initial pH displays a more acute intersection of the extrapolation of its two linear branches, with consequent improvement in the definition of the end point. For this reason it is preferable, though not essential, that the original pH setting be made closer to 6.5 than to 5.5.

Table I. Amperometric Titration of Fluoride

(Unless otherwise indicated, fluoride, as sodium fluoride, was dissolved in 100 ml. of 0.1 M potassium chloride and titrated with 0.1 M lead nitrate at room temperature in the presence of air, with the dropping electrode at a potential of −1.0 volt vs. the saturated calomel electrode; drop time, 3 to 6 seconds)

Trial No.	Fluoride. Mg.	Volume 0.1 M Pb(NO₃)₂ Theory	Exptl.	Error, %	Remarks
pH 5.2					
1	10.22	5.38	4.95	−8.0	...
2	11.31	5.95	5.62	−5.5	
pH 5.5					
3	9.91	5.21	5.20	−0.2	
4	10.09	5.31	5.33	+0.4	...
5	11.31	5.95	5.96	+0.2	
pH 6.5					
6	11.31	5.95	5.96	+0.2	
7	11.31	5.95	5.95	0.0	...
8	11.31	5.95	5.96	+0.2	
9	11.31	5.95	5.96	+0.2	
10	5.11	5.39 (0.05 M)	5.40 (0.05 M)	+0.2	Sample in 50 ml. of soln.
11	11.25	5.92	5.90	−0.4	Soln. deaerated
12	29.51	15.53	15.61	+0.5	
13	86.82	45.69	45.07	−1.3	0.15 M KCl
14	9.50	5.00	5.00	0.0	Soln. 0.01 M in Na₃PO₄; pptd. with AgNO₃
15	9.50	5.00	5.01	+0.2	
16	9.50	5.00	5.02	+0.4	
17	11.45	6.03	6.03	0.0	
18	11.62	6.11	6.13	+0.4	Soln. 0.1 M in KNO₃
19	9.64	5.07	5.08	+0.2	
20	10.77	5.67	5.55	−2.1	NaCl replaced KCl; soln. 0.5 M in NaClO₄
pH 7.0					
21	10.34	5.44	5.43	−0.2	...

All pH determinations were made with a glass electrode pH meter, but in later trials pH adjustment to a greenish hue of bromothymol blue proved adequate. Since the working range is at least 1 pH unit in breadth, indicator methods provide sufficiently accurate pH control. In the usual volumetric method the range within which the pH must be controlled is only 0.1 pH unit wide (4); also, the pH of about 4.65 recommended for the precipitation of lead chlorofluoride in the presence of an acetic acid–acetate buffer and a threefold excess of lead is much too low for this amperometric titration.

As the lead nitrate titrant was added, the pH of the titration mixture gradually moved toward lower values as the fluoride ion was replaced with nitrate. This does not cause any aberrations in the results, and since attempts to buffer the solution were un-

successful, owing to the tendency of lead to form insoluble precipitates or tight complexes with most of the common buffering agents, the pH was allowed to vary freely after the original setting. In their experiments Haul and Griess found that even when the conditions were such that the first separation of lead chlorofluoride from the solution was slow, further increments of reagent produced rapid precipitation of additional chlorofluoride. Consequently, if the original conditions produce rapid separation of a well formed lead chlorofluoride precipitate, the conditions during the latter part of the titration should not be as critical. With the samples used in most of this work (25 mg. of sodium fluoride in 100 ml. of solution), the final pH values were 4.7 and 5.4 when the original pH settings were 5.5 and 6.5, respectively. Results with a fluoride sample, three times as great as usual and handled at normal dilution (100 ml.) and an initial pH of 6.5, were 0.5% in error (trial 12). For best results, larger quantities of fluoride should be diluted until the concentration approximates that of the present work. However, when a sample nine times as large as usual was run at 100-ml. dilution, the results were only slightly more than 1% in error, although the final pH of this titration mixture was 4.0 (trial 13).
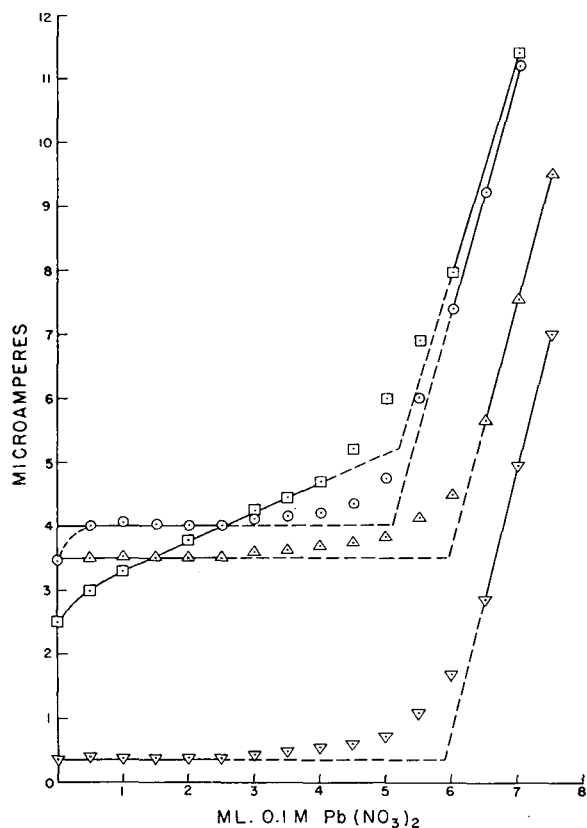

Figure 1. Amperometric Titration Curves

△ Trial 7, pH 6.5
⊙ Trial 19, pH 6.5, 0.1 M KNO₃ added
▽ Trial 11, pH 6.5, solution deaerated
⊡ Trial 3, pH 5.5 (different dropping electrode)

The reasons for the relatively sharp delimitation of the optimum range of initial values of pH, between 5.5 and 6.5, can only be conjectured. Since at pH 5 only 1% of the fluoride present is bound as hydrogen fluoride or HF_2^-, it is not clear how this factor could be responsible for the substantial delay observed in the approach to equilibrium even during the early stages of a titration made at this pH. It seems more probable that when the pH is too low, the more extensive adsorption of hydrogen ion on the surface of an initially colloidal lead chlorofluoride precipitate tends to stabilize the suspension, delaying its conversion to a

massive precipitate of minimum solubility. If this redistribution were delayed, the progressive change in solubility accompanying it would provide an explanation for the prolonged downward drift of the amperometric readings obtained in the early stages of a titration performed in excessively acid solution.

The alkaline boundary is probably more easily explicable in terms of incipient local precipitation of lead hydroxide. At pH 7 the hydroxide concentration is not high enough to precipitate lead from the bulk of the solution in which its concentration never exceeds a few millimoles per liter. However, the hydroxyl concentration is high enough to produce transient precipitation at the point at which the 0.1 M lead nitrate titrant is added to the solution. The slow conversion of this precipitate to the chlorofluoride would account for the more poorly defined readings found in solutions with a pH exceeding 6.5. Local precipitation of lead hydroxide may also occur in a region where the hydroxyl concentration is increased by the outward diffusion of hydroxyl ion from the dropping electrode, at which it is formed during the reduction of the oxygen in the undeaerated solutions.

There is no closely defined upper limit to the amount of fluoride that can be determined by this method if the sample is suitably diluted. A minimum concentration of about 0.1 mg. of fluoride per ml. of solution was used in these experiments, and as the titrations can be carried out with a microburet and a volume of titration solution no greater than 5 ml. (6), as little as 0.5 mg. of fluoride can be determined by this method with an accuracy of 0.5% or better. The fluoride requirement is, thus, less than one twentieth of that needed for the usual volumetric procedure (4), and the present method complements Langer's procedure (6), which is applicable to much lower concentrations.

The chloride concentration is a noncritical parameter, provided that it remains in the general vicinity of 0.1 M. For the quantities of fluoride used in most of this work this represents a twenty-fold excess. In the analysis of an abnormally large fluoride sample in the presence of 0.15 M chloride, the results obtained were only slightly higher than 1% in error, although there was present only a threefold excess of chloride. The low results may be due to the formation of a little lead fluoride under these conditions.

The presence of oxygen in the solutions appeared to cause no difficulties. A titration curve obtained with intermittent deaeration of the titration mixture is shown in Figure 1. All the current readings are diminished, but the shape of the curve and the position of the extrapolated end point are unaffected. It was concluded that deaeration was unnecessary.

Anionic interferents precipitated by silver ion are easily removed with this reagent. After filtration, the small excess of silver is precipitated with potassium chloride, the solution is again filtered, and the titration may be concluded as usual. Sulfate, which forms an insoluble salt with lead, but not with silver, is detrimental. Cations like aluminum and titanium which form tight fluoride complexes also interfere, but can be removed by preliminary treatment of the solution (2). Special interferences and/or aberrations accompanying the application of this method to various insoluble mineral and ceramic substances should be investigated under the particular conditions of the special case.

The effect of ionic strength was first investigated by the addition of sodium nitrate. When the titration solution was 0.1 M in this salt the results were of normal accuracy, but at higher concentrations anomalous effects were observed. Although catalytic reduction of the nitrate at the dropping electrode may be responsible for a minor part of this aberration, the major cause appears to be the existence of a lead nitrate complex ion. In support of this conclusion, a solution 5 millimolar in fluoride ion and 0.1 M in chloride was made 1 M in sodium nitrate; no precipitate was formed when small amounts of lead nitrate were added to the solution. When the conditions were unchanged except by the substitution of 1 M sodium perchlorate for the nitrate, precipitation was instantaneous. Even in concentrations of nitrate as low as 0.25 M the precipitation of lead chlorofluoride was inordi-

nately delayed and noticeably incomplete. According to Hume (*3*) the existence of a lead nitrate complex of appreciable stability is strongly indicated.

Abandoning the use of sodium nitrate, the ionic strength of the titration solution was increased by the addition of sodium perchlorate. When the concentration of this material was increased to 0.5 M an error of 2% was observed. Thus, results of optimum accuracy can be obtained only in solutions of moderately low ionic strength, but since the method works well at low fluoride concentrations, it will usually be sufficient to dilute a solution of high ionic strength.

An accuracy somewhat better than 0.5% can be maintained under the optimum conditions defined above. The ampero-metrically determined end point coincides with the stoichiometric equivalence point within the experimental error.

LITERATURE CITED

(1) Haul, R., and Griess, W., *Z. anorg. Chem.*, **259**, 42 (1949).
(2) Hoffman, J. I., and Lundell, G. E., *Bur. Standards J. Research*, **3**, 581 (1929).
(3) Hume, D., private communication.
(4) Kaufman, S., ANAL. CHEM., **21**, 582 (1949).
(5) Kolthoff, I. M., and Lingane, J. J., "Polarography," New York, Interscience Publishers, 1941.
(6) Langer, A., IND. ENG. CHEM., ANAL. ED., **12**, 511 (1940).
(7) Rinck, E., *Bull. soc. chim.*, **1948**, 305.

RECEIVED March 10, 1950.

Spectrophotometric Study of the Ruthenium-Thiourea Complex

GILBERT H. AYRES AND FREDERICK YOUNG

The University of Texas, Austin, Tex.

A spectrophotometric study has been made of the blue color produced when solutions of ruthenium(IV) or ruthenium(III) chloro complexes are treated with acid and thiourea. In hot solutions containing hydrochloric acid and ethyl alcohol the color develops rapidly and is stable. The system has a sharp absorption band at 305 mμ, and a broad absorption band at 620 mμ. The latter wave length was chosen as more suitable for use, primarily from the standpoint of avoiding interference from other metals. A photometer with suitable light filter could be used satisfactorily. When transmittancy measurements are made at 620 mμ with a Beckman spectrophotometer using 1-cm. cells, the optimum concentration range for the measurement is about 2 to 15 p.p.m. (micrograms per milliliter) of ruthenium. Maximum attainable accuracy for the photo-metric process is 2.7% relative error per 1% absolute photometric error, or about 0.5% relative error for a precision of 0.2% in making the measurements. Of the other platinum metals, only palladium and osmium interfere at 620 mμ; a concentration of 7 p.p.m. of ruthenium will tolerate about 0.7 p.p.m. of palladium and about 0.2 p.p.m. of osmium. Interference tests were made with solutions of iron, cobalt, nickel, copper, and chromium; of these, only cobalt and chromium give appreciable interference. By the usual methods employed for separation of ruthenium and osmium from all other metals and from each other, cation interference can be eliminated. Anions that might be introduced during the various separation methods—namely, bromide, hypochlorite, nitrate, sulfate, and perchlorate—do not interfere in the concentrations studied.

THE increasing commercial importance of ruthenium is well indicated by the patent literature on its uses in high density alloys, alloys for jewelry and pen points, in spark plug electrodes, switch contacts, and resistance wires, and as a catalyst. These uses suggest the need for a rapid, accurate method for determining ruthenium.

Many of the previous methods of analysis are tedious and/or subject to considerable error. Precipitation by replacement from acid solution by active metals gives a metallic "black" with high adsorptive properties which usually contains some of the base metal used as reagent (*21*, p. 711). Separation as sulfide followed by ignition yields oxide residues which are not of sufficiently exact composition for weighing, and some sulfur is always retained (*10*, p. 285; *21*, p. 710); ignition at too high a temperature can result in loss of ruthenium. Precipitation as hydrous oxide can be made from faintly alkaline solution (*24*), or from solutions of pH 6 (*9*), followed by ignition to oxide and reduction by hydrogen; the hydrous oxide is difficult to coagulate and filter, and may be considerably contaminated by coprecipitation of other substances. Ruthenium can be precipitated with thionalide (β-aminonaphthalide of thioglycollic acid) followed by ignition and reduction in hydrogen (*18*); for semimicro quantities, the results tend to be low by as much as 10% (*8*). A titrimetric method involving reduction of ruthenium(IV) to ruthenium(III) with tin (II) chloride (*13*) gave results which were always somewhat low.

Numerous color reactions of ruthenium have been reported (*17, 22, 23, 25, 26*); some of these reactions are applicable only to spot-test and microscopic identification, and are unsuitable for colorimetric determination on account of formation of insoluble products. Many color reactions, however, should be suitable for spectrophotometric determination of ruthenium; very little work along this line has been published. A reported colorimetric method for ruthenium, based on the "dark" color of its solution in hydrochloric acid to form H_2RuCl_5 (*27*), would be subject to considerable interference from other platinum metals in solution. Breckenridge and Singer (*5*) studied 5-hydroxyquinoline-8-carboxylic acid as a colorimetric reagent for ruthenium; spectral curves were presented, and the effect of the other platinum metals was investigated. Sandell (*20*) determined small amounts of osmium on the basis of the rose-red color produced by thiourea; Ayres and Wells (*3*) made a detailed spectrophotometric study of the osmium-thiourea system, including the effect of the blue color of ruthenium-thiourea as an interference.

The work of DeFord (7), which appeared late in 1949, gave an extensive bibliography of ruthenium, and included a study of the ruthenium-thiourea system. He gave methods for the application of the color reaction to ruthenium of various oxidation numbers and states of combination. DeFord's specification of optimum concentration range, corresponding to optical densities from 0.043 to 0.430, is in error (2). No data were given for interference from other colored metallic ions.

Table I. Standardization of Ruthenium Solutions

Soln. No.	Aliquot taken, ml.	Weight of ruthenium, g.	Concn. of solution, p.p.m. Ru	Colorimetric, Concn. of Solution, P.P.M. Ru
		Gravimetric		
1	100.0	0.0202	202	
	100.0	0.0203	203	
	100.0	0.0195	195	
		Av.	200	200[a]
2	50.0	0.0178	356	Used as spectro-
	100.0	0.0351	351	photometric standard
		Av.	354	
3	250.0	0.0232	93	94[b]

[a] Average of 12 separate determinations, with standard deviation of 0.6 p.p.m.
[b] Duplicates.

It is the purpose of the present investigation to make a spectrophotometric study of the ruthenium-thiourea color system, particularly with reference to evaluation of optimum range and maximum accuracy of the photometric process (2), and also to investigate the nature and extent of possible interference from other platinum metals and other common colored cations, as well as anions that might be introduced into a ruthenium solution when it is prepared for analysis. Another paper presents a similar study of the ruthenium–dithio-oxamide system (4).

REAGENTS

Ruthenium metal powder was obtained from the Fisher Scientific Company and from the American Platinum Works. Spectrographic examination of the samples showed absence of other platinum metals.

Test solutions of the other platinum metals, and the 10% thiourea solution, were prepared as described by Ayres and Wells (3). Test solutions of iron(III), cobalt(II), nickel(II), chromium(III), and copper(II) were prepared from the chloride salts; chromium(VI) was used in the form of potassium dichromate. Test solutions of bromide, hypochlorite, nitrate, sulfate, and perchlorate were prepared from their alkali salts.

APPARATUS

Transmittancy measurements were made with a Beckman Model DU spectrophotometer, using Corex cells of 1.004-cm. light path. The instrument was operated at constant sensitivity, using slit widths of the order of 0.02 to 0.10 mm., corresponding to nominal band widths of about 1 to 4 mμ.

In some of the preliminary work on the development of the method and testing for interference, spectral curves were made with a General Electric recording spectrophotometer.

EXPERIMENTAL

Preparation and Standardization of Ruthenium Solutions. Ruthenium metal powder, 0.2 to 0.8 gram, was treated under reflux condenser with a boiling mixture of 100 ml. of 5% sodium hypochlorite solution and 50 ml. of 2 M sodium hydroxide. The exit of the reflux condenser was connected to a trap containing sodium hydroxide solution to absorb any ruthenium tetroxide if it should volatilize from the mixture. Negative test for ruthenium in the trap solution confirmed the findings of Howe and Mercer (14) that no ruthenium tetroxide distills from alkaline hypochlorite solutions. The cooled hypochlorite solution was then rapidly acidified with hydrochloric acid, boiled to remove chlorine, cooled, and diluted to known volume; the final solution contained the ruthenium in the form of its chloro complexes, in 6 M

hydrochloric acid. During boiling to remove chlorine, considerable ruthenium tetroxide was lost by volatilization.

Attempts to standardize the ruthenium solutions by reduction with metals (10, pp. 274–5) either gave inconsistent results or were too tedious to be practical. Zinc and magnesium reductions produced very finely divided residues which adhered to the walls of the vessels and always contained some of the reductant metal. Iron (reduced powder) gave good precision as a reductant; however, at least five extractions of the residue with 6 M hydrochloric acid were required to remove all of the iron and iron salts. In all cases of metallic reduction, a residue of inconstant composition was produced, necessitating reduction by hydrogen before weighing.

Precipitation with thionalide (18), using samples containing about 20 mg. of ruthenium, gave extremely poor precision resulting from the rather high solubility of the precipitate; ruthenium could always be detected both in the mother liquor and in the wash solution.

The ruthenium solutions were successfully standardized by the method of Gilchrist and Wichers (9), in which precipitation from hot acid solution was effected by adding sodium bicarbonate to a pH of 6. Exercise of great care was necessary during filtration and washing, to prevent peptization of the precipitate. The oven-dried precipitate was reduced by heating at 700° C. for 20 to 30 minutes in a hydrogen atmosphere, and finally weighing as metal. The results of six individual determinations on three different solutions are shown in Table I. The standard curve (Figure 2) was constructed from data obtained by the use of aliquots of solution 2. Spectrophotometric comparison of the other solutions with solution 2 gave the results shown in the last column of Table I.
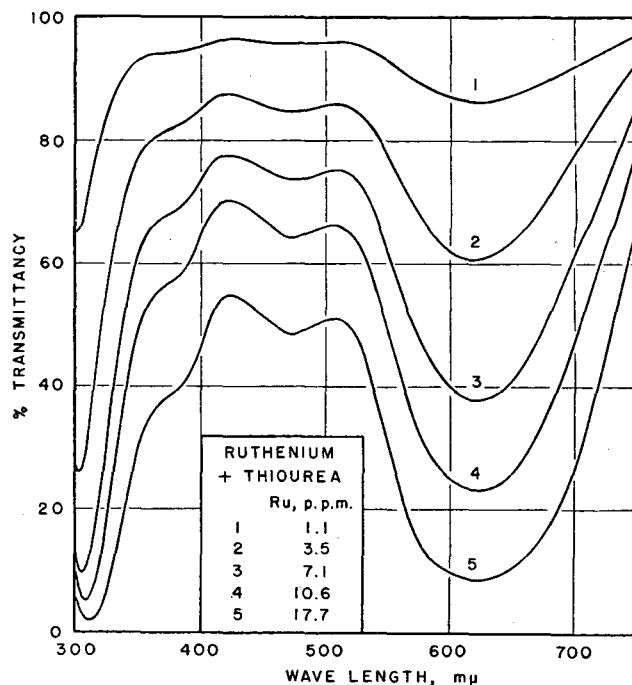

Figure 1. Spectral Transmittancy Curves for Ruthenium with Thiourea

Development of Color. By a preliminary study of the variables involved the following conditions were established as advantageous for the rapid development of a stable colored system: solution about 6 M in hydrochloric acid for initial color development; large excess of thiourea; presence of ethyl alcohol to the

extent of 50% by volume (to increase the rate of color formation and give a more stable color); heating at 85° C. (at much higher temperatures considerable alcohol was lost unless refluxing was used; at lower temperatures the rate of color development was slow); final solution about 4 M in hydrochloric acid. The procedure was as follows:

Appropriate aliquots of the stock standard solution, to give the final concentration desired, were added to 40 ml. of a 1 to 1 (by volume) mixture of concentrated hydrochloric acid and ethyl alcohol; after the addition of 5 ml. of 10% thiourea solution, the mixture was heated for 10 minutes in a water bath at 85° C. The solution was cooled, and made up to 100.0 ml. with a 1 to 1 mixture of 6 M hydrochloric acid and ethyl alcohol. Blanks contained the same amounts of reagents.

Data for transmittancy versus wave-length curves were obtained by measuring the transmittancy at frequent wave-length intervals over the range 750 to 300 mμ. Typical spectral curves for various ruthenium concentrations are shown in Figure 1. All transmittancy curves have a broad, flat minimum at 620 mμ; centered at about 480 mμ, the curves have a broad region of high transmittancy; a sharp inversion at 305 mμ is of somewhat lower transmittancy than the 620 mμ minimum. Below 305 mμ the transmittancy rises sharply. A plot of log transmittancy (620 mμ) against concentration showed good agreement with Beer's law over the range investigated (up to 18 p.p.m.).

Table II. Tolerance of Ruthenium-Thiourea Solution for Metal Interferences

(All solutions, 7.1 p.p.m. ruthenium)

Interfering Substance	Visual Color Produced by Interfering Substance Plus Reagents	Amount Tolerated, P.P.M.	% Interference Relative to Ruthenium
Osmium(IV)	Rose-red	0.2	3
Palladium(II)	Yellow	0.7	10
Iron(III)	Amber	5	70
Cobalt(II)	Blue	0.5	7
Nickel(II)	Green	20	280
Copper(II)	Greenish-yellow	5	70
Chromium(III)	Green	1	14

Rate of Color Development. When the concentrations of reagents specified in the previous section were used, color development at room temperature was very slow and reached a stable maximum only after a few days. When heated at 85° C. the mixtures developed to a maximum, stable color intensity within 10 minutes.

Stability of Color. Solutions containing various concentrations of ruthenium, developed by the procedure given previously, showed no measurable change in transmittancy over a period of 24 hours, and a change of only 0.2 to 0.4% (absolute) in 48 hours. A black precipitate formed in the more concentrated solutions after about 48 hours.

Reproducibility. A statistical treatment was made of the transmittancy measurements on 32 samples developed as described previously; the data, collected over a period of several weeks, included all analysis errors accumulating onward from the use of the stock standard solution, and no results were rejected. The standard deviation (σ) of the 32 measurements was 0.21 ± 0.02% absolute transmittancy (see discussion for the corresponding relative analysis error). On this basis, random discrepancies in transmittancy of 0.4% (2σ) or less can be expected with a probability of 95%.

Effect of Diverse Ions. Qualitative tests on solutions of the other platinum metals with thiourea showed that no color reaction was given by rhodium, iridium, and platinum, but that osmium produced a red solution and palladium produced a yellow solution. These results confirmed the findings of Ayres and Wells (3) in a spectrophotometric study of the osmium-thiourea system; their results indicated that interference with the ruthe-

nium-thiourea system might be expected from osmium and palladium. Interference from colored ions such as Ni^{++}, Co^{++}, Fe^{+++}, Cr^{+++}, Cr$_2$O$_7^{--}$, and Cu^{++} might be expected.

In order to determine the extent of interference by the ions mentioned, solutions containing a constant amount of ruthenium (7.1 p.p.m., which is in the optimum range for measurement) and varying amounts of the ion in question were developed with thiourea in the usual way. The interfering substance was added in the same state as that in which it would be found in solution prepared from the metal by the same treatment as used in dissolving the ruthenium. Thus, the common metals used would be present as iron(III), cobalt(II), nickel(II), copper(II), and chromium(VI) (dichromate). In the high concentrations of alcoholic hydrochloric acid used to develop the ruthenium-thiourea color, copper gave the characteristic greenish-yellow color of the chloro complex, and cobalt gave the blue color of the chloro and/or ethyl alcohol complex. Chromium(VI) was immediately reduced to chromium(III) when thiourea was added to the acid-alcohol mixture; iron(III) appeared not to be reduced by this treatment; osmium(VIII) reduced to osmium(IV) (chloro-osmate) and was complexed by the thiourea (3). Transmittancies of the ruthenium solutions containing the interfering substance were measured at wave lengths between 700 and 500 mμ; no shift of transmittancy minimum was observed. The tolerance of the ruthenium-thiourea system for the interfering substance was taken as the largest amount of that substance which would give a transmittancy, at 620 mμ, not more than 0.4% (absolute) different (3) from that of the ruthenium alone. The results of the interference tests on the various metals are shown in Table II, along with the color of the interfering substance after treatment with the reagents.
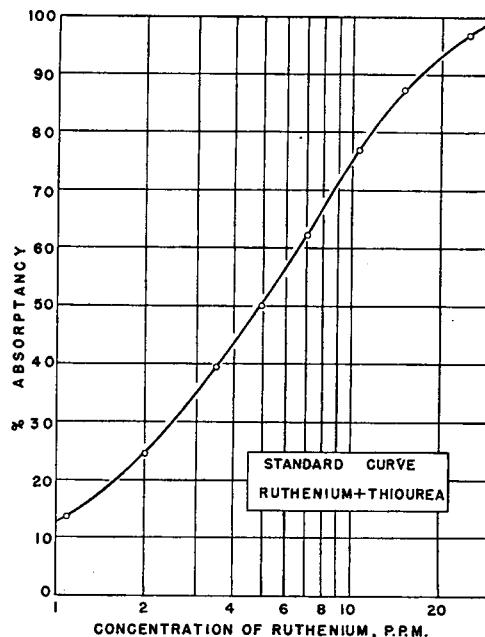

Figure 2. Calibration Curve for Ruthenium with Thiourea at 620 mμ

By the usual separation procedures for osmium and ruthenium from the other platinum metals and from each other, osmium is first distilled as osmium tetroxide from nitric acid solution. Before the separation of ruthenium, the nitric acid in the residual material is removed by repeated evaporation with hydrochloric acid and fuming down with sulfuric acid. After adding sodium bromate, ruthenium is distilled as RuO$_4$ (9). When absorbed in hydrochloric acid, ruthenium tetroxide gives a solution containing

ruthenium(IV)and/or ruthenium(III)chloro complexes(6,15). If the ruthenium tetroxide is absorbed in hydrochloric acid saturated with sulfur dioxide, excess of the latter is removed by boiling the acid mixture before proceeding with the analysis (9). If absorbed in a mixture of hydrochloric acid and ethyl alcohol, ruthenium(III) chloro complex is formed (14); this same complex is probably formed as an intermediate by the use of the acid-alcohol mixture in the color development process of the present study. If alkali is used to absorb ruthenium tetroxide, alkali ruthenate (Na_2RuO_4) is formed (12, 14), which on acidification with hydrochloric acid gives chloro complexes of ruthenium(IV) and/or ruthenium(III). After separation of osmium as OsO_4, ruthenium can be distilled as ruthenium tetroxide from perchloric acid solution (16). When ruthenium metal is attacked by alkaline oxidizing fluxes (1, 12, 19), solution of the melt in water gives ruthenate. From alkali hypochlorite dissolution, ruthenium can be distilled as ruthenium tetroxide in a current of chlorine (14).

Considering all these methods of attack and of separation of ruthenium, the only common anions besides chloride that could have been introduced in analytical amounts in the distillate are: bromide, from reduction of free bromine by sulfur dioxide; sulfate, from oxidation of sulfur dioxide; nitrate, from incomplete removal of nitric acid before distillation; hypochlorite, from incomplete removal of this reagent or by hydrolysis of chlorine; and perchlorate. The study of anion interference was therefore limited to these anions. In solutions containing 4 p.p.m. of ruthenium, all the anions mentioned above were without effect up to 100 p.p.m., hence were not studied at higher concentrations.

DISCUSSION

The calibration curve for the determination of ruthenium with thiourea is shown in Figure 2, in which per cent absorptancy (100 − % transmittancy) at 620 mμ is plotted against log concentration; each experimental point was established by many replicate measurements. The utility of this plotting method for evaluation of maximum attainable accuracy and suitable working range has been reported previously (2). The curve has its maximum slope at about 63% absorptancy, in agreement with Beer's law, hence a maximum accuracy corresponding to 2.7% relative analysis error per 1% absolute photometric error. The accuracy of the photometric process at any point can be evaluated graphically from the calibration curve by dividing 230 by the slope of the curve, expressed as the change in per cent absorptancy per logarithmic cycle (tenfold concentration change) of a tangent at the given point. By this method, a tangent to the curve at its steepest slope covers a change of 85% absorptancy for one logarithm cycle of abscissa; the maximum accuracy is therefore 230/85 = 2.7% relative analysis error per 1% photometric error. The maximum accuracy occurs at about 7 p.p.m. of ruthenium. Inspection of the calibration curve shows that the error is not much greater between 2 and 15 p.p.m., which would be a satisfactory working range of concentration.

If desired, the limits of the optimum range can be defined more exactly; suppose, for example, that it is desired to find the concentration range within which the photometric error will not exceed 4% relative error per 1% absolute photometric error; inasmuch as replicate samples can be reproduced to 0.2% absolute transmittancy, this would represent a relative analysis error of 0.8%. A relative error of 4% per 1% photometric error corresponds to a slope of 230/4 = 58% absorptancy change per log cycle on the concentration axis. The upper and lower concentration limits are easily determined graphically by translating a slope of 58 to the calibration curve, and noting the point of tangency at the low and at the high portion of the curve, and the corresponding concentrations. By this method, the concentration limits are between 3 and 13 p.p.m. of ruthenium, in good agreement with the range of 2 to 15 p.p.m. roughly estimated by inspection. The concentration range can be extended upward, with some increase in accuracy, by the use of the differential method of measurement (2, 11).

A calibration curve plotted from the 305-mμ transmittancies is

parallel to the 620-mμ curve shown in Figure 2, but is displaced toward lower concentrations to an extent such that the optimum range is about 0.6 to 5 p.p.m. of ruthenium. For this absorption band a slight shift of the wave length of the transmittancy minimum from 300 to 310 mμ was noted as the concentration of ruthenium increased.

The spectral curve centering around 620 mμ has a broad minimum, and between 610 and 630 mμ the transmittancy changes only a few tenths of a per cent; hence, narrow band widths of incident light are not required, and filter photometers would be suitable for making the measurements.

The temperature coefficient of transmittancy of the ruthenium thiourea system is so small (about 0.05% absolute transmittancy per 1° C., and reversible) that no difficulty is experienced from temperature variations of a few degrees.

The rather low tolerance of the ruthenium-thiourea system for osmium is no special disadvantage in analysis, because sharp separations of osmium from ruthenium can be made by appropriate distillation procedures (9, 14).

LITERATURE CITED

(1) Antony, V., and Lucchesi, A., *Gazz. chim. ital.*, **29**, ii, 321 (1894).
(2) Ayres, G. H., ANAL. CHEM., **21**, 652 (1949).
(3) Ayres, G. H., and Wells, W. N., *Ibid.*, **22**, 317 (1950).
(4) Ayres, G. H., and Young, Frederick., *Ibid.*, **22**, 1281 (1950).
(5) Breckenridge, J. G., and Singer, S. A. G., *Can. J. Research*, **25B**, 49 (1947).
(6) Charonnat, R., *Ann. chim.*, **16**, 5 (1931).
(7) DeFord, D. D., "Chemistry of Ruthenium," Oak Ridge, U. S. Atomic Energy Commission, *Document* **NP-1104** (Dec. 9, 1949).
(8) Flagg, J. F., "Organic Reagents Used in Gravimetric and Volumetric Analysis," p. 281, New York, Interscience Publishers, 1948.
(9) Gilchrist, Raleigh, and Wichers, Edward, *J. Am. Chem. Soc.*, **57**, 2565 (1935).
(10) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," New York, John Wiley & Sons, 1929.
(11) Hiskey, C. F., ANAL. CHEM., **21**, 1440 (1949).
(12) Hopkins, B. S., "Chapters in the Chemistry of the Rarer Elements," Chap. 21, p. 23, Champaign, Ill., Stipes Publishing Co., 1940.
(13) Howe, J. L., *J. Am. Chem. Soc.*, **49**, 2393 (1927).
(14) Howe, J. L., and Mercer, F. N., *Ibid.*, **47**, 2926 (1925).
(15) Mellor, J. W., "Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. XV, p. 520, London, Longmans, Green and Co., 1936.
(16) Noyes, A. A., and Bray, W. C., "A System of Qualitative Analysis for the Rare Elements," p. 40, New York, Macmillan Co., 1948.
(17) Ogburn, S. C., Jr., *J. Am. Chem. Soc.*, **48**, 2493 (1926).
(18) Rogers, W. J., Beamish, F. E., and Russell, D. S., IND. ENG. CHEM., ANAL. ED., **12**, 561 (1940).
(19) Saitō, Shoichirō, *Bull. Inst. Phys.-Chem. Research (Tokyo)*, **8**, 164 (1929).
(20) Sandell, E. B., IND. ENG. CHEM., ANAL. ED., **16**, 342 (1944).
(21) "Scott's Standard Methods of Analysis," Furman, N. H., ed., 5th ed., Vol. I, New York, D. Van Nostrand Co., 1939.
(22) Steiger, B., *Mikrochemie*, **16**, 193 (1935).
(23) Whitmore, W. F., and Schneider, H., *Ibid.*, **17**, 279 (1935).
(24) Wichers, E., *J. Am. Chem. Soc.*, **46**, 1829 (1924).
(25) Wohler, L., and Metz, L., *Z. anorg. Chem.*, **138**, 368 (1924).
(26) Yoe, J. H., and Overholser, L. G., IND. ENG. CHEM., ANAL. ED., **14**, 435 (1942).
(27) Zvyagintzev, O. E., *J. Applied Chem. (U.S.S.R.)*, **4**, 1085 (1931).

Spectrophotometric Study of the Ruthenium–Dithio-oxamide Complex

GILBERT H. AYRES AND FREDERICK YOUNG

The University of Texas, Austin, Tex.

The blue color produced when solutions of ruthenium(IV) or ruthenium(III) chloro complexes are treated with dithio-oxamide (rubeanic acid) has been studied spectrophotometrically. In hot solutions containing hydrochloric acid and ethyl alcohol the color develops rapidly, and is stable for 24 to 48 hours. Measured at 650 mµ against a reagent blank with a Beckman spectrophotometer (1-cm. cells), best accuracy is obtained when solutions contain about 2 p.p.m. (micrograms per milliliter) of ruthenium; in this region the relative analysis error is 2.7% per 1% absolute photometric error, or about 0.8% relative error for a precision of 0.3% (standard deviation) in the photometric process. In the range 1 to 5 p.p.m. of ruthenium the relative error does not exceed 1%. Interference tolerances are given for other platinum metals, common cations which are colored, and anions that might be present in certain separation procedures. Osmium is the only platinum metal that interferes extensively at 650 mµ; hence a sharp prior separation of osmium, if present, is required for determination of ruthenium.

IN A previous paper (2) the authors presented a spectrophotometric study of the ruthenium-thiourea color system, evaluated the optimum range and maximum accuracy of the photometric process, and determined the tolerance of the system for certain interfering substances.

Among the various color reactions of ruthenium that have been reported, it appeared that the blue reaction product with dithio-oxamide (rubeanic acid) (5, 6) would be suitable for spectrophotometric determination. It is the purpose of this investigation to make a study of the ruthenium–dithio-oxamide reaction to define the best conditions for color development, to evaluate the optimum range and the accuracy of the photometric process, and to determine the nature and extent of interferences, especially of other platinum metals.

REAGENTS AND APPARATUS

The standard ruthenium solution [ruthenium(IV) and/or ruthenium(III) chloro complexes], and the solutions of the various cations and anions used in the determination of interferences were the same as those employed in the study of the ruthenium-thiourea system (2).

Dithio-oxamide, Eastman chemical No. 4394, was used in the form of a 0.2% solution in glacial acetic acid.

Transmittancy measurements were made, in Corex cells of 1.004-cm. light path, with a Beckman Model DU spectrophotometer, operated at constant sensitivity, using slit widths of the order of 0.02 to 0.10 mm., corresponding to band widths of about 1 to 4 mµ.

EXPERIMENTAL

Development of Color. Preliminary experiments showed that a high concentration of acid was required, and that a large amount of ethyl alcohol was desirable to prevent formation of precipitates and to give a more stable color system. At room temperature the color developed very slowly (over a period of a few days), but at elevated temperatures developed rapidly.

Appropriate aliquots of the stock standard solution, to give a final concentration of 0.3 to 8 p.p.m. of ruthenium, were treated with 40 ml. of 1 to 1 (by volume) mixture of concentrated hydrochloric acid and 95% ethyl alcohol, and 15 ml. of the dithio-oxamide reagent. The mixture was heated on a water bath at 85° C. for 30 minutes, and was then cooled and made up to 100 ml. with a 1 to 1 mixture of 6 M hydrochloric acid and ethyl alcohol. Blanks contained the same amounts of reagents.

Spectral Characteristics. Data for the spectral curves were obtained by measuring the transmittancy at frequent wavelength intervals over the range of 800 to 350 mµ. Curves for several concentrations of ruthenium are shown in Figure 1. The curves have a very broad transmittancy minimum at 650 mµ, a maximum at 460 mµ, and a second sharp minimum at about 370 mµ, below which the transmittancy again rises sharply; the second minimum showed a shift toward longer wave lengths as the concentration of ruthenium increased. A plot of log transmittancy, at 650 mµ, against concentration showed good agreement with Beer's law; transmittancy values at the minimum centered about 370 mµ were not in good agreement with Beer's law.
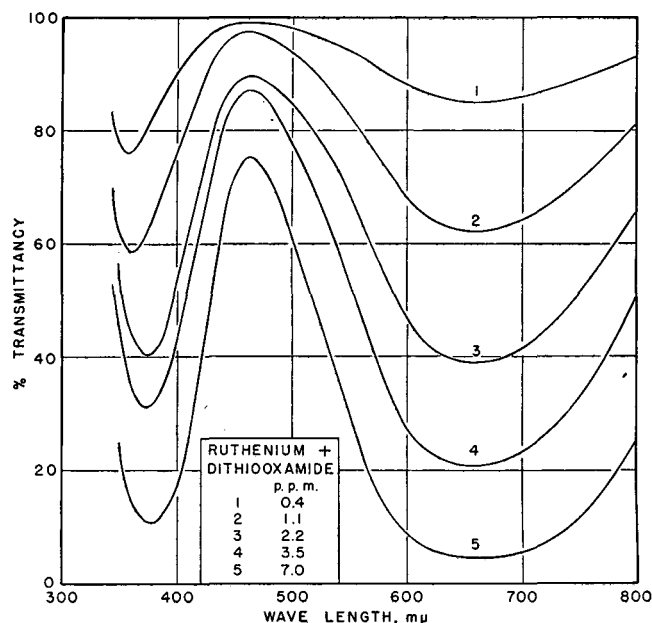

Figure 1. Spectral Curves for Ruthenium with Dithio-oxamide

Stability of Color. Solutions containing various amounts of ruthenium showed no measurable change in transmittancy in 24 hours, and a change of only about 0.4% (absolute) in 48 hours. On longer standing a black precipitate separated. This behavior was essentially identical with that found for the ruthenium-thiourea system (2).

Effect of Temperature. In the range 25° to 40° C. the effect

of temperature was found to be about +0.03% absolute transmittancy per 1° C.

Reproducibility. A statistical treatment was made of the transmittancy measurements on 47 samples (2.2 p.p.m. of ruthenium, transmittancy 39.3%), developed as described previously. The data, collected over a period of several weeks, included all analysis errors accumulating in the procedure from the point at which the aliquot of the standard solution was taken; no results were rejected. The average deviation was 0.23% and the standard deviation was 0.30% absolute transmittancy.

tion of ruthenium. The curve has maximum slope at about 63% absorptancy, in agreement with derivations from Beer's law (1). Graphical evaluation of maximum photometric accuracy gives a value of 2.7% relative error per 1% absolute photometric error, or about 0.8% relative error, on the concentration measured, for a photometric error of 0.3% (the standard deviation previously mentioned); this accuracy is attainable at a concentration of about 2 p.p.m. of ruthenium. In order to keep the relative error within 1%, the concentration must be within the limits 1 to 5 p.p.m. These limits were evaluated as follows: Assuming the photometric error to be 0.3% absolute, a relative error of 1% on the concentration measured would correspond to 3.4% relative error per 1% absolute photometric error. This error corresponds to a slope of 230/3.4 = 68% absorptancy change per log cycle on the concentration axis—i.e., a tenfold concentration change. A line having this slope is tangent to the calibration curve at about 1 p.p.m. on the lower portion and at about 5 p.p.m. on the upper portion of the curve. The range can be extended to higher concentrations, with an increase in accuracy, by comparison of a sample solution with a reference standard instead of a blank (1, 3).

A plot of per cent absorptancy at 370 mμ (the second minimum) against log concentration of ruthenium gives a curve almost parallel to the curve shown in Figure 3, but displaced slightly toward

Table I. Tolerance of Ruthenium for Interfering Substances

Interfering Substance	Dithio-oxamide Method (2.2 P.P.M. Ruthenium)			Thiourea Method (7.1 P.P.M. Ruthenium)		
	Visual color, interfering substance + reagents	Tolerance, p.p.m.	% relative to ruthenium	Visual color, interfering substance + reagents	Tolerance, p.p.m.	% relative to ruthenium
Osmium (IV)	Olive green	0.05	2	Rose red	0.2	3
Rhodium (III)	Orange	0.5	23	Colorless	No interference	
Iridium (IV)	Yellow	0.9	41	Colorless	No interference	
Palladium (II)	Yellow	10	455	Yellow	0.7	10
Platinum (IV)	Orange red	2	91	Colorless	No interference	
Iron(III)	Amber	5	230	Amber	5	70
Cobalt(II)	Blue	2	91	Blue	0.5	7
Nickel(II)	Light green	1	45	Light green	20	280
Copper(II)	Greenish yellow	2	91	Greenish yellow	5	70
Chromium[a]	Green	5	230	Green	1	14

[a] Chromium(VI) was reduced to chromium(III) by reagents.

Effect of Diverse Ions. Solutions of the other platinum metals were developed with dithio-oxamide in the usual way, and their spectral curves determined in order to predict what interference might be expected. These curves, shown in Figure 2, indicate that at 650 mμ the measurements for ruthenium should not be subject to appreciable error from moderate amounts of the other platinum metals except osmium. At the second ruthenium minimum, 370 mμ, palladium and rhodium would interfere seriously; other platinum metals would interfere to a considerable extent.

The extent of interference by the other platinum metals, as well as by the common colored cations, was determined by measuring the transmittancies of color-developed solutions containing a constant amount of ruthenium (2.2 p.p.m., which is in the optimum range for measurement) and varying amounts of the interfering ion. Transmittancy measurements were made over a considerable wave-length region on either side of 650 mμ; no shift in the minimum was observed. The tolerance of the ruthenium–dithio-oxamide system for the interfering substance was taken as the largest amount of that substance which would give a transmittancy not more than 0.4% absolute different from that of the ruthenium alone. Table I lists the interfering substances tested, their individual colors after treatment with the reagents, and the tolerance as defined above. For comparison, colors and tolerances for the ruthenium-thiourea method (2) are included.

In a previous article (2) it was shown that by the use of various procedures for the dissolution and/or separation of ruthenium, the only anions (beside chloride) that could be present in analytical amounts are bromide, sulfate, nitrate, hypochlorite, or perchlorate; the study of anion interference was therefore limited to these anions. In solutions containing 2.2 p.p.m. of ruthenium, all these anions are without effect up to 100 p.p.m., hence were not studied further.

DISCUSSION

In the calibration curve, Figure 3, per cent absorptancy (100 − % transmittancy) at 650 mμ is plotted against log concentra-

higher concentration. However, measurements of unknowns at 370 mμ would be unsatisfactory on account of interference from other platinum metals.

During the course of making measurements for the spectral curves for osmium–dithio-oxamide, a striking anomaly was encountered. When measured at once, after development in the usual way, the transmittance of the solutions (containing 5 and 10 p.p.m. of osmium) was very much higher than that of the blank solution in the region between about 365 and 400 mμ. In this region the transmittancy was changing very rapidly with time during the first hour or more, and reached a stable value only
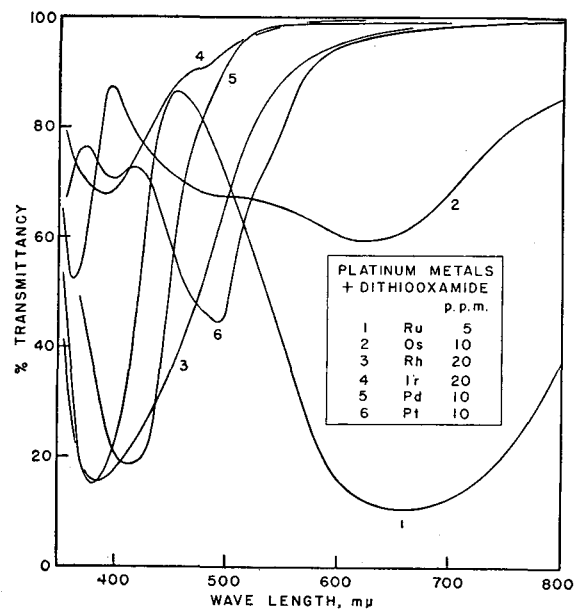

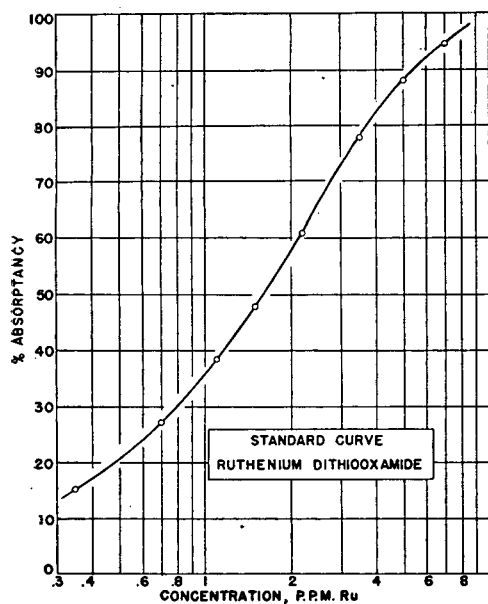Figure 2. Spectral Curves for Platinum Metals with Dithio-oxamide

Figure 3. Calibration Curve for Ruthenium with Dithio-oxamide, 650 mμ

after several hours. Between 450 and 800 mμ, stable readings were obtained from the start. After about 2 hours the transmittancies in the lower wave-length region had undergone a reversal, so that the transmittancy of the 5 p.p.m. solution was considerably lower than that of the 10 p.p.m. solution; the spectral curves for the two concentrations crossed at about 410 mμ. Above 450 mμ the two curves were still parallel in their proper relation to concentration and at the same transmittancies as when first measured. This behavior was checked throughout; no explanation has been found as yet, and the effect should be studied further. The osmium curve shown in Figure 2 was plotted from data taken about 2 hours after color development.

Wölbling and Steiger (6) found that palladium and platinum salts yield red precipitates with dithio-oxamide. These precipitates form only when palladium and platinum are present in con-

siderably higher concentrations than were required in the study of interferences in the present investigation. Welcher (4), apparently referring to the work of Wölbling and Steiger, states, "It is important to know that osmium, which in its other analytical reactions resembles ruthenium, does not react with rubeanic acid; for this reason rubeanic acid can be used for the detection of ruthenium in the presence of osmium." The original article by Wölbling and Steiger states, "Osmium salt solutions give, with rubeanic acid, no essential color change; with large amounts, the ethyl acetate extract is brown." In the present work, no extraction procedures with organic solvents were used. Rather large amounts of ethyl alcohol and strong acid, and elevated temperatures, were required to give rapid development of the blue ruthenium–dithio-oxamide color; under these conditions osmium gave a color; when the reagents were mixed the solution assumed a greenish color, which on heating changed rapidly to a brownish red, then more slowly to an olive green color. By the proposed method, therefore, reasonably sharp prior separation of osmium from ruthenium, by an appropriate distillation procedure (2), would be required.

Compared with the thiourea method, the dithio-oxamide method has a slightly lower optimum concentration range, although the difference in ranges is hardly large enough to be of importance in application to analyses. The choice between the two methods probably would be based upon the kind and amount of interfering substances present in the sample.

LITERATURE CITED

(1) Ayres, G. H., ANAL. CHEM., 21, 652 (1949).
(2) Ayres, G. H., and Young, Frederick, Ibid., 22, 1277 (1950).
(3) Hiskey, C. F., Ibid., 21, 1440 (1949).
(4) Welcher, F. J., "Organic Analytical Reagents," Vol. IV, p. 154, New York, D. Van Nostrand Co., 1948.
(5) Wölbling, H., Ber., 67B, 773 (1934).
(6) Wölbling, H., and Steiger, B., Mikrochemie, 15, 295 (1934).

Determination of Sodium Monoxide in Sodium

### LEONARD P. PEPKOWITZ AND WILLIAM C. JUDD

Knolls Atomic Power Laboratory, General Electric Company, Schenectady, N. Y.

THE initial attempt by the authors to devise a method for the determination of sodium monoxide in sodium was to use the water formed by the reaction of sodium monoxide with dry hydrogen chloride at high temperatures. The water formed immediately reacts with the excess sodium to form sodium hydroxide, which is reduced by the sodium at the elevated temperatures to reform sodium monoxide (4).

$$2NaOH + 2Na \longrightarrow 2Na_2O + H_2 \qquad (1)$$

In all such reactions the total sodium sample must react completely before the water equivalent to the oxygen present will be released. The determination of the small amounts of water produced in the presence of the very large quantity of sodium chloride is as difficult a task as the primary determination. Such exploratory attempts made without success included reactions with dry hydrogen chloride or carbon tetrachloride in liquid

sodium–vapor phase reactions at elevated temperature and in dry benzene with iodine, bromine, and hydrogen chloride. Dry glacial acetic acid has some promise as a reagent, if a method could be developed for detecting the small quantity of water formed and if the glacial acetic could be dried reproducibly.

$$2Na + 2HAc \longrightarrow 2NaAc + H_2 \qquad (2)$$

$$Na_2O + 2HAc \longrightarrow 2NaAc + H_2O \qquad (3)$$
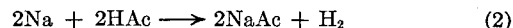
The measurement of the water conductometrically appeared to be very sensitive but nonreproducible because of the original variable water content of the glacial acetic acid.

The simple method reported in this paper depends on the physical separation of sodium from the sodium oxide by repeated extractions with mercury. The sodium oxide is insoluble in the resulting sodium amalgam and floats on the surface of the amalgam. Following the extraction, the sodium monoxide is dis-

A method is presented for the determination of sodium monoxide in sodium. The basis of the procedure is the extraction of the metallic sodium with mercury in a special apparatus, effecting a quantitative separation from the sodium monoxide. The mean deviation obtained is ±0.005% oxygen. A novel sampling procedure is described which is an integral part of the method.

solved in water and titrated to a phenolphthalein end point, or alternatively the sodium equivalent to the oxygen is determined with a flame photometer. The sample size is determined by titration of the separated sodium amalgam. From these two determinations the percentage of oxygen in the sample can be calculated.

The wash water must be corrected for the carbon dioxide content. In practice the wash water is carefully neutralized to a phenolphthalein end point before use. This has given more reproducible results than titrating to a methyl orange end point.

At room temperature, oxygen can exist in sodium as sodium hydroxide, sodium monoxide, sodium peroxide, or sodium carbonate. However, at higher temperatures sodium hydroxide (4) and sodium peroxide are reduced to sodium monoxide by sodium. Repeated attempts to detect carbonate in sodium which had been heated to approximately 400° C. by dissolving the sodium in acid and measuring the carbon dioxide liberated indicated that no carbonate was present in the sample investigated. Accordingly if the sodium to be analyzed is heated to 400° C. before sampling, sodium monoxide is the only compound that need be considered. This simple titration procedure will not measure the oxygen present in the sodium as zinc oxide, magnesium oxide, or any compound which is insoluble in sodium amalgam or mercury and which is not titratable to a phenolphthalein end point. If any other titratable oxide is involved in the titration, it will be included in the calculation as sodium monoxide.
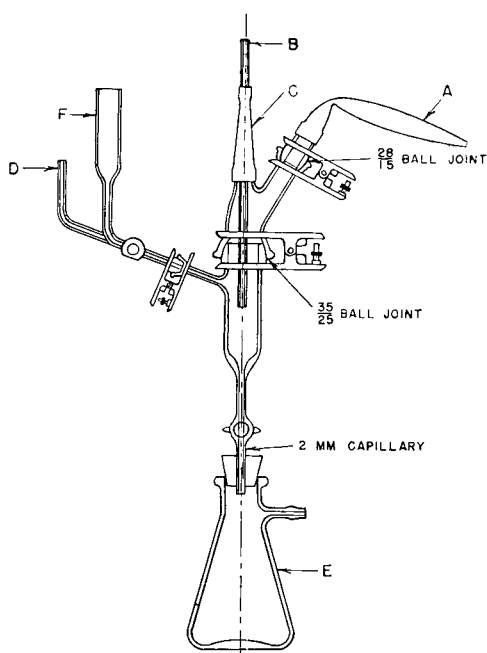

Figure 1. Extraction Apparatus

Although a number of the results reported in this paper were obtained by the titration procedure, the above difficulty can be eliminated by determining the sodium equivalent to the oxygen by means of a flame photometer (1), using lithium as an internal standard (see Table V).

PREPARATION OF SAMPLE

One of the most difficult problems in any procedure for the determination of oxygen in sodium is an adequate and reliable sampling technique. The described extraction procedure allows for a simple solution to the sampling difficulties.

The samples are taken by drawing up the molten sodium (125° C.) from a container into standard 0.25-inch (0.6-cm.) borosilicate glass tubing, 12 inches (30 cm.) long. The sodium is allowed to solidify and the tube containing the sample is brought out into the air. The sodium-filled tubes are cut into 3-inch lengths (ca. 2 grams of sodium), thus affording three aliquots of the sample. The ends of the 3-inch lengths are sealed with de Khotinsky cement to prevent the oxide particles, formed during exposure to the air, from contaminating the equipment during the subsequent handling. The 3-inch length is deeply scored about 0.75 inch from each end, taking care not to break through the walls of the tubing, and the sample is thoroughly wiped and dried. There has been no evidence of further diffusion of oxygen down the sodium column even after many weeks of exposure. This observation has also recently been reported by Dostrovsky and Llewellyn (2).

Samples of sodium distilled in vacuum into small glass capsules which were sealed under vacuum gave identical results (0.01% oxygen) as samples of the same sodium distilled into 0.25-inch tubing and treated as in the above sampling procedure. For much of the preliminary investigation these small sealed bulbs (1 to 2 grams) of triple-distilled sodium were used as described below.

APPARATUS

The extraction apparatus is shown in Figure 1. A is a length of Gooch rubber tubing, which is closed at one end and cemented and wired to a male 28/15 semiball joint at the other. The sodium sample contained in the glass tube previously described is placed in A. The stirring rod, B, which is connected to the extraction chamber through the gas-tight flexible rubber sleeve, C, is used to break the glass sample tubes, stir the amalgam, and scrub down the walls of the extraction chamber. The dry, inert gas (nitrogen or argon) enters the extraction chamber through the side arm, D. The vacuum flask, E, is the receiver for the sodium amalgam and is replaced by a similar flask during the solution of the sodium oxide. The mercury is introduced into the extractor through funnel F, which is mounted on the gas inlet tube.

The gas purifying system is visible in Figure 2, which is an over-all photograph of a unitized assembly for two extraction chambers. The tank gas, argon or nitrogen, is passed through a reducing valve and a safety valve into a drying tube filled with Anhydrone. From the drying tube the gas passes over freshly reduced copper contained in two furnaces, as shown in Figure 2, at a temperature of 400° C. to remove the oxygen and then over Anhydrone to remove any residual moisture. The gas is led to the two extractors through a manifold visible in Figure 2.

PROCEDURE

Preparation of Extractor. The extractor, after thorough cleaning and drying, is attached to the gas manifold by means of D (Figure 1) and an asbestos-paper wrapped clamp (Figure 2). All joints and stopcocks are lubricated with silicone stopcock lubricant.

The 3-inch length of tubing containing the sodium, after being wiped with a moist cloth and then with a dry cloth, is flamed carefully to remove the last traces of moisture and placed in sampling tube A (Figure 1). After the cap and stirring rod B have been flamed, the apparatus is assembled and dried by heating with a torch during the subsequent flushing and evacuation.

The air in the extractor is removed by alternate flushings with nitrogen and evacuation. Approximately 50 such cycles, which take less than 5 minutes, will remove the air from the extractor and provide a sufficiently satisfactory inert atmosphere. During this interval the extractor is thoroughly flamed with a torch to remove any moisture which may have condensed within the

apparatus. When the extractor is dry and free of oxygen, triple-distilled mercury is poured into F and about 20 ml. are run into the extractor.

The front and back ends of the sample tube are broken off inside A at the scratches and the pieces containing the contaminated sodium are retained in A. The middle portion of the sample tube containing the uncontaminated sodium is dropped into the extraction chamber.

Table I. Recoveries of Oxygen Added as Sodium Monoxide to 1-Gram Samples of Triple-Distilled Sodium

Oxygen Added %	Oxygen Found %	Deviation %
0[a] (av. of 3 determinations)	0.02	
0.07	0.08	+0.01
0.07	0.07	0
0.08[b]	0.07	−0.01
0.19[c]	0.19	0
0.22[d]	0.23	+0.01
0.24	0.28	+0.04
0.29	0.34	+0.05
0.64	0.63	−0.01
1.14	1.09	−0.05
		Av. ±0.02

[a] Blank values due to Na₂O content of triple-distilled sodium. Blank value has been subtracted from succeeding recovery values.
[b] Time of separation 15 minutes.
[c] Time of separation 45 seconds.
[d] Time of separation 4 hours.

Extraction. A positive gas pressure, distending the rubber tubing, is maintained throughout the subsequent extraction steps. The sample is pushed under the mercury in the extraction chamber and the glass tubing is thoroughly broken up with the stirring rod. In order to withstand the shock of tapping on the stirring rod, the bottom of the extractor is thickened as indicated in Figure 1. The mercury amalgamates vigorously with the sodium and some amalgam may be thrown onto the walls of the extractor. Considerable heat is evolved and the extractor is cooled with an air blast while being stirred vigorously to complete the amalgamation. When cool, the amalgam is drawn into the suction flask by applying a very slight suction. The amalgam is withdrawn until 1 to 2 ml. remain in the extractor to retain the oxide. If the amalgam is completely removed, a loss of sodium monoxide will occur.

The extractions are repeated until the extractor, stirring rod, and glass fragments are free from any metallic luster. About eight to ten cycles are usually required to remove all the sodium. When the extraction is thought to be complete, the lower stopcock is closed and the flask containing the amalgam is replaced with a fresh flask containing 5 ml. of water and a few drops of phenolphthalein. Another extraction is performed and the mercury is drawn into the suction flask. If no sodium is present in the mercury, the indicator will remain colorless and the extraction is complete. If there is some residual sodium, the extractions are repeated; the amalgam is added to the original flask until all the sodium is removed.

Following the complete removal of the sodium, the extractor cap is removed and the stirring rod is washed with distilled water, which is used to dissolve the sodium oxide in the extractor. The resulting sodium hydroxide is drawn into a clean suction flask. The extractor is washed quantitatively and the resulting solution is titrated with 0.005 N hydrochloric acid to a phenolphthalein end point to determine the hydroxyl ion equivalent to the sodium monoxide in the sample

$$\text{Mg. of oxygen} = \text{ml. of HCl} \times 0.005 \times 8.00 \qquad (4)$$

If the flame photometer is used, 100 p.p.m. of lithium are added as an internal standard (1), and the solution is filtered through a coarse sintered-glass filter with a layer of Supercel as the actual filter medium and made to 100 ml. The instrument is used as directed by the manufacturers (5).

In order to determine the sample size, an excess of standard hydrochloric acid is added to the separated sodium amalgam with vigorous stirring and is back-titrated with standard sodium hydroxide to a phenolphthalein end point. There is a rapid evolution of hydrogen in this step, with attendant fire hazard.

EXPERIMENTAL RESULTS AND DISCUSSION

For convenience of comparison and interpretation of experiment, the analytical results are calculated as per cent oxygen and are reported as such in the tables instead of per cent sodium monoxide (% Na₂O = % oxygen × 3.88).

In order to test the method, weighed amounts of sodium monoxide were added to a bulb of triple-distilled sodium in the extraction chamber, the bulb was broken under the mercury, and the amalgam was stirred vigorously to disperse the added sodium monoxide. The sodium monoxide was standardized by dissolving in water and titrating with standard hydrochloric acid to a phenolphthalein end point. Because the recovered sodium monoxide was measured in an identical manner, the original titer value can be used to calculate the recovery, despite the fact that the sodium monoxide used was not pure but contained some sodium hydroxide.

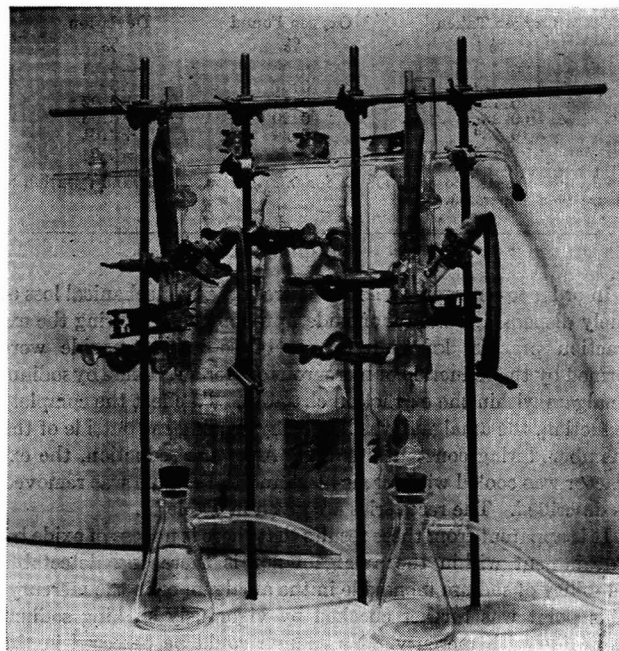The recovery data given in Table I indicate the effectiveness of the method.

Figure 2. Over-all View of Apparatus

It was determined experimentally that, at least in times of the order of 45 seconds, there was no loss of oxide by solution or entrainment in the amalgam. The amalgam was removed within 45 seconds after formation, followed by a second addition of mercury which was also removed within 45 seconds. The remaining sodium was extracted as usual. The experiment was repeated with intervals of 15 minutes and 4 hours. The data obtained are included in Table I.

Table II. Recoveries of Known Amounts of Oxygen

(Supplied as HgO or Hg₂O after reduction with sodium amalgam)

$$2Na(Hg) + HgO \rightarrow Na_2O + Hg$$

Oxygen Taken %	Oxygen Found %	Deviation %
0.005 (HgO)	0.007	+0.002
0.009 (HgO)	0.011	+0.002
0.014 (HgO)	0.015	+0.001
0.032 (HgO)	0.028	−0.004
0.035 (Hg₂O)	0.041	+0.006
0.11 (HgO)	0.11	0
0.17 (Hg₂O)	0.15	−0.02
		Av. ±0.005

In order to determine whether the simple phenolphthalein test indicated the complete removal of sodium, the following experiments were performed. Because the residual oxides are dissolved in water, any sodium that may be present will evolve an equivalent amount of hydrogen. The hydrogen, if present, was swept over hot copper oxide and the resulting water was absorbed and

weighed. Known amounts of hydrogen were generated within the extractor by dissolving weighed amounts of magnesium ribbon in hydrochloric acid added through the side arm. Quantitative recoveries of the hydrogen released in this manner were obtained. However, no hydrogen due to residual sodium was ever detected following the extraction; this indicated the complete removal of sodium from the oxides and the validity of the simple phenolphthalein test for the completeness of extraction.

Table III. Recoveries of Known Amounts of Oxygen

(Supplied as HgO after reduction with sodium in sealed capsules)

Oxygen Taken	Oxygen Found	Deviation
%	%	%
0.05[a]	0.04	−0.01
0.07[a]	0.07	0
0.12	0.14	+0.02
0.23	0.20	−0.03
0.23[a]	0.24	+0.01
		Av. ±0.01

[a] Samples heated to ca. 200° C. for 0.5 hour with constant agitation to ensure good dispersion.

In order to determine whether there was any mechanical loss of finely dispersed sodium monoxide in the amalgam during the extraction process, known amounts of sodium monoxide were formed by the reduction of mercurous or mercuric oxide by sodium amalgam within the extraction chamber. To effect the complete reduction, the amalgam was heated by flaming the outside of the chamber during constant stirring. After the reduction, the extractor was cooled with an air blast and the sodium was removed as described. The recoveries are given in Table II.

It is apparent from these results that there is no loss of oxide by entrainment within the amalgam nor is there any detectable solubility of sodium monoxide in the amalgam or in the mercury. This point was further checked by vigorously shaking sodium monoxide with mercury. No sodium could be detected in the mercury. This experiment was also performed at 200° C. with the same result.

Standard samples were prepared by distilling sodium into glass bulbs containing weighed amounts of mercuric oxide. The bulbs were sealed off and the sodium was melted by heating to ca. 125° C. to complete the reduction of the mercuric oxide. This procedure resulted in standard samples of sodium containing known amounts of oxygen with the resulting sodium oxide dispersed in the sodium. However, the extent of uniform dispersion is not known. The recoveries are given in Table III.

The data in Table IV indicate the precision that can be obtained with the method, using triple-distilled sodium as replicate samples.

In order to compare the results obtained by the flame photometer and titration methods and to substantiate the validity of the statement that only sodium monoxide need be considered under the described sampling conditions, the following experiments were performed. Following the titration, the lithium internal standard was added directly to the resulting neutralized solution. The solution was filtered, made to volume, and run in the flame photometer. The results are given in Table V.

The pickup of oxygen from glass by sodium is negligible at temperatures up to 200° C. for periods of an hour. In fact, the glass can have a very slight golden discoloration without a significant increase in the oxygen content. However, over extended periods of heating at 200° C. or at higher temperatures, there is a decided attack on the glass, which is indicated by a blackening of the glass with an accompanying increase in the oxygen content of the sodium. The normal oxygen content of triple-distilled sodium is approximately 0.01%, but after the sealed glass capsule had been heated to 400° C. for an hour, the oxygen content was 0.5%.

The solubility of sodium in mercury at room temperatures is 0.7% by weight (3). Approximately 20 ml. (272 grams) of mercury are used for each extraction; therefore (272)(0.007) = 1.9 grams of sodium could theoretically be removed in one pass. In actual practice eight to ten passes are made to wash down the sodium and amalgam thrown up on the walls of the extraction chamber during the amalgamation.

The mechanism of the separation involved is entirely analogous to any extraction procedure where one component (sodium) is extracted by a solvent (mercury), and the second component (sodium monoxide) is not extracted by the solvent. The great difference in density (Hg = 13.6, Na_2O = 2.27) of the two substances adds to the efficiency of the process, as does the negligible solubility of the oxide in the amalgam and the anomalous low viscosity of mercury (H_2O at 20° C. = 0.0101 poise, mercury at 200° C. = 0.0102 poise).

Within the limitations in obtaining representative and reproducible samples of sodium, the mean deviation of the method is ±0.005% oxygen. The theoretical precision of the method is determined only by the precision of the final sodium determination. However, in actual practice because the method is essentially a physical separation, the precision and accuracy are strongly dependent on technique and experience. The lower limit is set, at present, by the mean deviation. An experienced operator can easily obtain a mean deviation of ±0.005% oxygen on triplicate aliquots from the same sample. By the same token, there is no maximum limit except that set by volumetric considerations.

Table IV. Oxygen Content of Triple-Distilled Sodium

Oxygen	Deviation from Mean
%	%
0.004	−0.004
0.005	−0.003
0.005	−0.003
0.006	−0.002
0.008	0
0.008	0
0.010	+0.002
0.016	+0.008
Mean 0.008	±0.003

Table V. Comparison of Titration and Flame Photometer Methods

(Per cent oxygen in sodium)

Titration	Flame Photometer	Deviations
0.004	0.004	0
0.005	0.004	−0.001
0.006	0.008	+0.002
0.007	0.011	+0.004
0.008	0.009	+0.001
0.012	0.011	−0.001
		Av. ±0.002

The application of this technique to other systems in which the oxide is not soluble in the resulting amalgam is obvious. Such a system is zinc–zinc oxide. An investigation of the determination of zinc oxide in zinc by the present method is planned for the future.

LITERATURE CITED

(1) Berry, J. W., Chappell, D. G., and Barnes, R. B., IND. ENG. CHEM., ANAL. ED., 18, 19 (1946).
(2) Dostrovsky, I., and Llewellyn, D. R., J. Soc. Chem. Ind., 68, 208 (1949).
(3) Hansen, M., "Der Aufban der Zweistofflegierungen," Berlin, Julius Springer, 1936.
(4) Pepkowitz, L. P., and Proud, E. R., ANAL. CHEM., 21, 1000 (1949).
(5) Perkins-Elmer Corp., "Instruction Manual, Model 52A," 1948.

Colorimetric Estimation of Tetrachloronitrobenzene

M. E. AUERBACH

Sterling-Winthrop Research Institute, Rensselaer, N. Y.

A sensitive colorimetric method is described for the determination of tetrachloronitrobenzene, the active ingredient of an agricultural dust. Color formation probably depends upon the interaction of tetrachloronitrobenzene with acetone, in the presence of tetraethylammonium hydroxide.

EXPERIMENTS conducted within recent years indicate that 2,3,5,6-tetrachloronitrobenzene (TCNB) may become an important agent for the control of dry rot (*Fusarium coeruleum*) in potatoes (*1*) and for the simultaneous suppression of sprouting in stored potatoes (*2, 3*). In field tests, the material has been applied in the form of a dust containing 3% of the active substance mixed with an inert carrier, such as kaolin. It would be of obvious value to food supply or health authorities to have available a sensitive test method for the pure chemical which might eventually be adapted to the assay of residual tetrachloronitrobenzene on potatoes intended for human consumption. A quantitative colorimetric test has been worked out in these laboratories.

Pure 2,3,5,6-tetrachloronitrobenzene is a colorless (or very faintly yellow) odorless crystalline material which melts at 99° to 101° C., corrected. It is practically insoluble in water, moderately soluble in alcohol or petroleum ether, and readily soluble in ethyl ether, chloroform, and benzene.
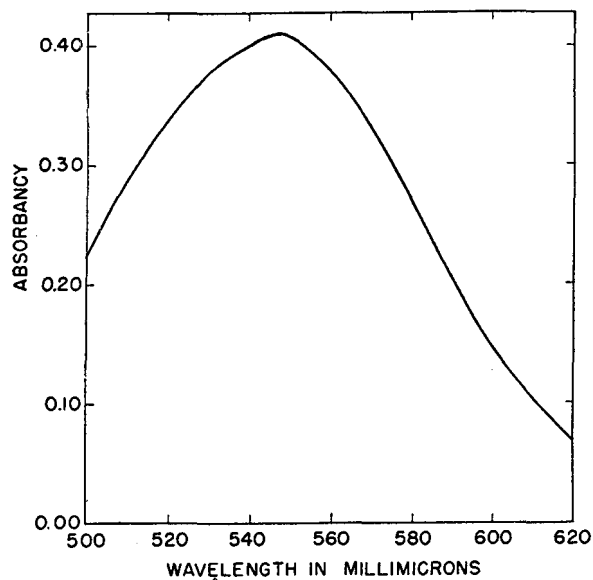

Figure 1. Absorption Spectrum of Colored Derivative of Tetrachloronitrobenzene

60 micrograms in 10 ml.

The labile nature of the chlorine ortho to the nitro group, and the presence of the nitro group itself, suggested the possibility of a color test based on the intense color of the nitroquinoid ion, as developed, for example, in the well-known Janovsky or Zimmermann test using *m*-dinitrobenzene (*4*). This turned out to be a fruitful approach.

MATERIALS REQUIRED

Tetraethylammonium hydroxide, 10%, available from Eastman Kodak Company, Catalog No. 2078.

Tetraethylammonium hydroxide working solution (2%). Dilute 2 ml. of the 10% tetraethylammonium hydroxide with 8 ml. of reagent grade acetone. The dilution should be used within 4 or 5 hours after preparation.

Standard tetrachloronitrobenzene solution. Dissolve exactly 100 mg. of tetrachloronitrobenzene in exactly 100 ml. of reagent grade acetone. From this stock solution, by appropriate dilution, prepare a working standard which will contain 10 micrograms of tetrachloronitrobenzene per ml. of acetone.

PREPARATION OF STANDARD CURVE

Into six test tubes accurately graduated with a mark at 10 ml., transfer, respectively, 0, 1, 2, 3, 4, and 5 ml. of the standard solution of tetrachloronitrobenzene (10 micrograms per ml.). Dilute the contents of each tube to exactly 10 ml. with reagent grade acetone, and mix. Add exactly 0.1 ml. of the 2% tetraethylammonium hydroxide to each tube, and mix. After 10 minutes, but before 20 minutes have elapsed after the addition of the quaternary base, read the standard series in a suitable photoelectric colorimeter, using a glass color filter which transmits a band of light in the range 540 to 560 mμ. When the colorimeter readings are plotted on semilog paper, a linear graph results. The spectral characteristics of the purplish-red color developed in the test were determined with a Beckman spectrophotometer, and are shown in Figure 1. A broad absorption band occurs in the range 536 to 556 mμ, with the peak at about 548 mμ.

GENERAL CONSIDERATIONS

As indicated, the color develops gradually, reaching maximum intensity after 7 or 8 minutes, and begins to fade after about 20 minutes. An excess of quaternary base decreases the sensitivity of the test and the purity of the color, and also hastens fading. Precautions must be taken to exclude more than minute amounts of water. The effect of water in the acetone solvent is shown in Table I.

Table I. Effect of Added Water on Color Developed in 10-Ml. Portions of Acetone Containing 20 Micrograms of Tetrachloronitrobenzene

% Water, by Volume	% T
0 (negligible)	71.7
0.55	78.7
1.10	90.2
1.65	95.9

Generally speaking, the admixture of other common solvents with the acetone should be avoided. An exception is benzene. A mixture of 4 volumes of reagent grade benzene and 6 volumes of acetone is entirely suitable, although with this mixture the color develops somewhat more slowly than in straight acetone. The mixture is important, because it would enable one to wash treated potatoes with a water-immiscible solvent (benzene) in which tetrachloronitrobenzene is extremely soluble. After filtering, the benzene wash might be diluted with 6 volumes of acetone, and assayed for tetrachloronitrobenzene directly. Acetone itself is not the most suitable wash solvent for treated potatoes, inasmuch as it would probably become hydrated in the process, and thus yield falsely low results.

1287

The constitution of the colored derivative of tetrachloronitrobenzene is thought to be:
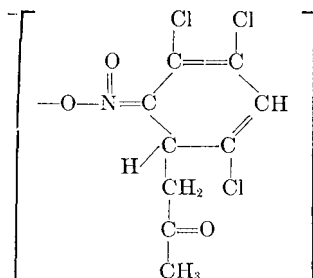
Another possibility is the bis compound, in which both chlorines ortho to the nitro group are eliminated through reaction with active hydrogen of the acetone reagent. However, no attempt has been made to prove the mechanism of color formation.

LITERATURE CITED

(1) Bayer Products, Ltd., London, Fusarex Technical Memorandum 3.
(2) Brown, W., *Ann. Applied Biol.*, 34, 422 (1947).
(3) Luckwill, L. C., "Suppression of Sprouting in Ware Potatoes," Annual Report, Agricultural and Horticultural Research Station, Long Ashton, Bristol, England, 1948.
(4) Zimmermann, W., *Z. physiol. Chem.*, 245, 47 (1936).

RECEIVED April 27, 1950.

Radiometric Titration with Radioactive Silver as End-Point Indicator

ALOIS LANGER[1]

Westinghouse Research Laboratories, East Pittsburgh, Pa.

Radiometric titrations were extended to include some argentometric titrations using radioactive silver as the indicator. A simplification of the titration procedure is described in which only two points on a branch of the titration curve are determined. The possibility of a radioactive exchange method for determining the amount of a given precipitate is discussed.

IT HAS been demonstrated (3) that it is possible to determine the end point of a precipitation reaction carried out stepwise as a titration by radioactivity measurements of the solution if it contains a proper radioactive element as an indicator. The substance to be titrated, the reagent, or both can be tagged by adding the radioactive element in tracer amounts directly to the solution or by incorporating it as a constituent—for example, in the reagent. Depending upon how the volumetric procedure is performed, different titration curves can be obtained which are quite similar to the ones known from conductometric, amperometric (polarographic), and other titrations, where the readings of the end-point indicator are directly proportional to the concentration of the ion in solution. Although generally limited to reactions in which insoluble precipitates are formed, a discussion of the basic titration curves (4) indicated the possibility of using some graphical means for determining the end point in cases of higher solubility for the reactions of the type $A^+ + B^- = AB$ as a precipitate. This discussion was restricted to the ideal cases where there is no dilution occurring during the titration or in which such an effect can be neglected.

The first trials of this method gave promising results by using radioactive phosphate as reagent in titrations of silver, lead, and a few other elements. Nevertheless the method was tested further by using radioactive silver as indicator in a few cases of known argentometric titrations. From this study, which confirmed previous findings, a simplification of the method evolved; this simplified method requires only the determination of the least number of points on one branch of the titration curve. Although such a simplified procedure is limited to cases where the solubility of the precipitate formed is sufficiently low, in order not to distort the results, it is, in view of the nature of radioactive determinations, a more promising method.

[1] Present address, Westinghouse Atomic Power Division, Pittsburgh, Pa.

APPARATUS AND PROCEDURE

The apparatus and procedure used were basically the same as in the previous investigation (3). The radioactivity of the solution was determined by specially constructed pipet counters for liquids. The different constructions tried during this and other investigations are illustrated in Figure 1. The inclined-jacketed counter, A, whose side arms are indicated by the broken lines, drains better than the vertical one and is probably the better one to use. In some cases the other counters can be used with advantage. Thus, for elements emitting γ radiation, arrangement B consisting of a dipping counter inserted in a hollow pipet can be utilized. For smaller amounts of liquids or for securing thinner windows when softer radiation has to penetrate, the front or side window counter with a permanently attached, C, or a detachable pipet, D, might be preferred. The pipet is in the form of a round disk. For small amounts of liquids below 0.5 ml. a counter, E, was constructed containing the liquid in a thin capillary; the outside of this capillary was platinized and thus served as the wire electrode of the counter. These counters expose their liquid content, with a constant layer thickness and the same solid angle, to the sensitive region of the counter; thus excellent reproducibility is attained. The spacing of the liquid layer can be chosen in relation to the self-absorption of the radiation in the liquid, in order to secure the highest counting yields with the minimum amount of solution. The closed construction enables a thorough shielding of the counter, eliminates contamination of the surroundings by spilling, and is easily adaptable to remote control.

In order to increase the accuracy of the radioactive determinations, the counting-rate meter was replaced by a mechanical counter with a scaling unit of 64. With the mechanical counter it is possible, with sufficient counting, to obtain more accurate data for weak activities than with the counting-rate meter observed visually. The probable percentage of error inherent in all counting measurements of radioactivity is $67/\sqrt{N}$ where N is the total number of counts taken. All necessary tests were made to secure proper operation of the instrument.

The method reported is essentially a micromethod using a 5-ml. microburet to titrate 50 ml. of solution, but any other scale can easily be adopted. The radioactive element is an indicator whose

sensitivity can be varied according to the problem under investigation.

The initial radioactivity of the solution can be made as high as the radioactivity level and the available amount of the radioactive element permit. However, in this work it was preferably kept in the range of a few thousand counts per minute, to avoid excessive losses in equipment and the necessity for applying corrections. For the radiation emitted from the 45-day half-life silver, absorption losses due to change in composition of the solution during titration are negligible.

In the titration procedure, after each addition of a discrete amount of reagent to the known volume of liquid, a definite portion is drawn into the counter envelope through a fritted glass plate. The porosity of the fritted plate can be chosen according to the consistency of the precipitate, but for some precipitates it was quicker to centrifuge first and then draw the clear liquid into the counter, thus avoiding the plugging of the porous plate and the consequent prolonging of suction and discharge time. The counting was done for a given time rather than for a given number of counts. The readings obtained were corrected for volume change during the titration owing to the addition of the reagent, by multiplying with a factor which is the ratio of the final volume, $V_0 + V$, to the original volume, V_0.

RESULTS WITH TITRATION METHOD

The radioactive silver was obtained by proton bombardment of palladium which, after addition of carrier silver, was electroplated from an ammonia solution. The silver was dissolved in nitric acid and a part of this highly radioactive solution was added to the stock solution of 0.01 M silver nitrate and the resulting reagent having the desired radioactivity was standardized by normal procedures. Some typical curves are indicated in Figure 2. Because the solubility of the silver halide precipitate is low, the titration curves deviate only slightly from straight lines. When radioactive bromide was added to the titrated sodium bromide, the characteristic V-shaped titration curve was easily obtained. It was found by straight titration of the separate halides that the reproducibility is about ±2%.

Preliminary results indicate that by using radioactive bromine with silver it should be possible to determine chlorine, bromine, and iodine together in one titration because of the different solubilities of the precipitates formed; a complicated titration curve would result. Further testing of the accuracy of such a procedure is necessary, because some rounding of the curves at the end points, probably due to coprecipitation, was observed.

Table I

A_0, Ag in 50 Ml. Soln., Mg.	B_1, NaCl Added, Ml. (1 Ml. = 20.6 Mg. Ag)	C_0, Counts/ Min.	C_1, Counts/ Min., Cor.	A_0, Ag Found, Mg.
53.6	0.75	4840	3448	53.5
53.6	1.00	4836	2976	54.6
53.6	1.50	4838	2100	53.9
53.6	2.00	4846	1156	53.7

A_0, Cl in 50 Ml. Soln., Mg.	B_2, AgNO$_3$ Added, Ml. (1 Ml. = 3.55 Mg. Cl)	B_3, AgNO$_3$ Added, Ml. (1 Ml. = 3.55 Mg. Cl)	C_2, Counts/ Min., Cor.	C_3, Counts/ Min., Cor.	A_0, Cl Found, Mg.
17.75	7.0	9.0	3002	6120	18.01
21.30	7.0	9.0	1410	3996	20.98
10.65	5.0	8.0	3068	7558	10.47
10.65	4.0	6.0	1486	4575	10.78

SIMPLIFICATION OF TITRATION PROCEDURE

The determination of many points on the radiometric titration curve is not a rapid procedure, especially if accuracy is desired for the points at low radioactivity of the solution. At least 5000 counts are required to secure a probable error of 1%. It is, therefore, an advantage to determine the end point from two measurements corresponding to two points on the titration curve and compute the amount of reagent needed to reach the end point algebraically. This procedure was used by Moeller and Schweitzer (5) in their investigation of thorium.

The shape of the basic titration curve used offers two possibilities. One case comes from the L- and V-shaped titration curve and the other one from the J- and V-type. The two possibilities are graphically indicated in Figure 2. For the first case let us suppose that C_0 is the initial radioactivity of the solution and that B_1 ml. of reagent is added in an amount that does not completely precipitate the substance from the solution and a remaining activity of C_1 is left in the solution. This activity, C_1, is preferably made so that $C_1 = C_0/2$, and if a completely unknown solution is present, the amount of reagent can quickly be determined by trial addition of the reagent and a preliminary counting. It can be shown, assuming an insoluble precipitate and correcting the readings for volume change, that:
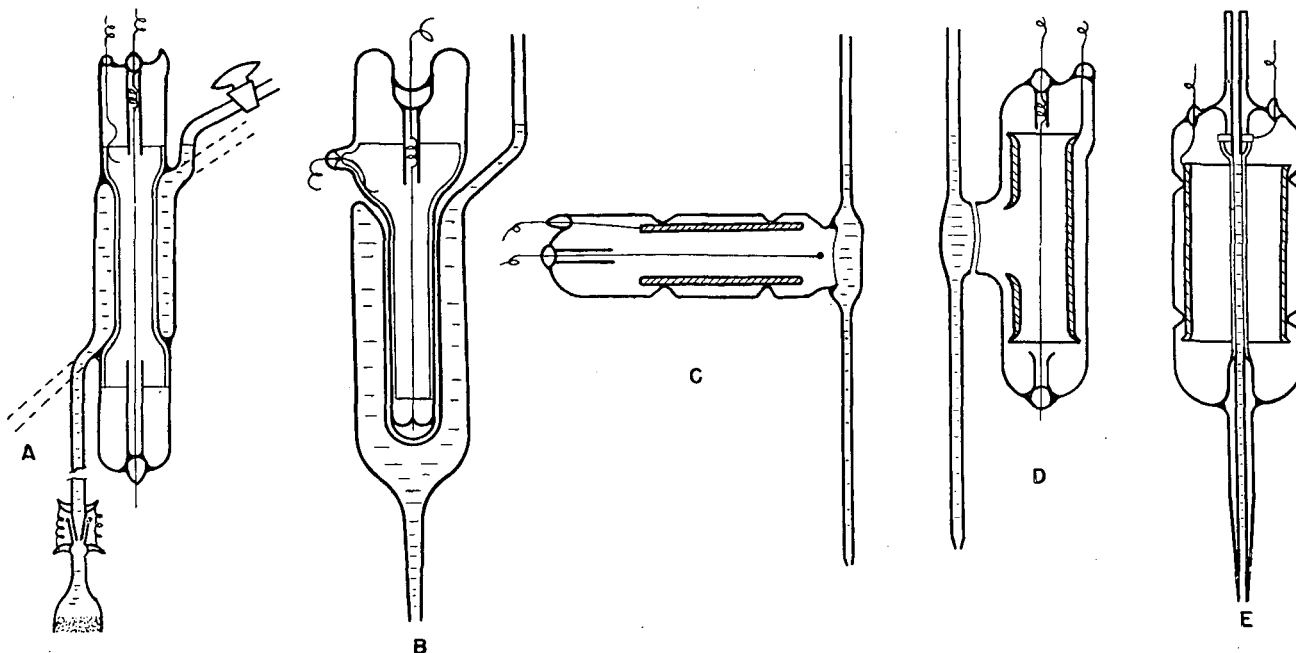

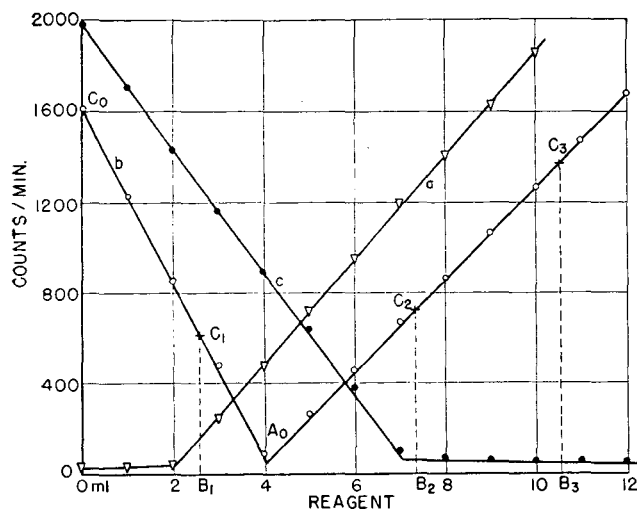**Figure 1. Counter Constructions for Liquids**

Figure 2. Typical Titration Curves in Starting Volume of 50 Ml.

a. Titration of 7.09 mg. Cl as NaCl with 0.1 M Ag*NO$_3$
b. Titration of 31.9 mg. Br as NaBr* with 0.01 M Ag*NO$_3$
c. Titration of 75.5 mg. Ag as Ag*NO$_3$ with 0.1 M NaBr

$$A_0 = B_1 \frac{C_0}{C_0 - C_1} \tag{1}$$

where A_0 is the amount of reagent needed to reach the end point of the titration curve or the amount of material to be determined. This procedure can be used with advantage to correlate the radioactive level of the solution, C_0, with the total amount of substance present, A_0.

In the second case of a J- and also a V-shaped titration curve where principally the added reagent is radioactive, an excess of reagent B_2 must first be added and the corresponding counting rate, C_2, of the solution obtained. After addition of more reagent, the total amount of which is B_3, the corresponding counting rate, C_3, is measured. In such a case A_0 is computed by the equation:

$$A_0 = \frac{B_2 C_3 - B_3 C_2}{C_3 - C_2} \tag{2}$$

This is actually the more important case of titration, where a nonradioactive substance is determined by a radioactive reagent. With V-type titration curves, in order to avoid errors, the further addition of a small amount of reagent will indicate on which branch of the titration curve the determination was made. The procedure of determining only two points is more readily adaptable to radiometric work, since values can be determined and computations carried out more accurately. The errors inherent in determining the end point by graphical means on a limited scale, which does not attain the accuracy of the buret reading or that of the concentration indicator, are not introduced. A few typical results obtained for the different halogens are summarized in Table I. In the given cases the results are as reproducible as those of a many-point titration procedure.

RADIOACTIVE EXCHANGE METHOD

When radioactive elements are added to a system containing a suspended precipitate, the radioactivity of the solution diminishes in time. In some cases when the same ions are in the solution as in the lattice of the precipitate the uptake of the radioactive atoms from the solution might be due to diffusion and recrystallization (*1, 6*). During a given time the radioactivity of the liquid approaches a constant level. The rate of approach is dependent on the particle size of the precipitate, the diffusion coefficient of the particular ion, the temperature of the system, and the rate of recrystallization. Constant radioactivity is reached

when a homogeneous distribution of the radioactive ions throughout the whole system, including the precipitate, has been established (*2*). For cases when this interchange of ions takes place rapidly, an analytical method similar to the isotope dilution method can be derived.

Assume we have A grams of atoms in a solid which is suspended and constantly shaken with a solution containing B grams of the same atoms, but labeled with some radioactive ones; let the counting rate of B in the solution, under standard conditions, be C_B, and the counting rate under the same conditions, when the time interval has elapsed to effect homogeneous spread, be C_{AB}. In all these cases the counting rate correlates the number of radioactive atoms to the nonactive ones. It can easily be shown that A is given by the equation:

$$A = B \left(\frac{C_B}{C_{AB}} - 1 \right) \tag{3}$$

Thus, from the added amount of B atoms and the counting rates C_B and C_{AB}, the original amount of A atoms is determined. The method differs from the isotope dilution method only in the sense that the mixing of the radioactive and nonactive species proceeds by diffusion and is ionic, when otherwise it is normally molecular and is accomplished by direct mixing. In the method of mixing by diffusion it must first be established empirically that the radioactive atoms are distributing themselves throughout the whole precipitate homogeneously and that the time of shaking is sufficiently long to reach the desired equilibrium because the rate of uptake is approaching the limit asymptotically. Also, corrections for decay must be applied if long shakings are necessary and dilution of the solutions has to be considered.

Some results obtained by the use of radioactive silver on silver halides are given in Table II.

Table II

(Fresh precipitates, time of shaking—30 min.)

A, Ag Used, Mg.	Compd.	B, Ag Added, Mg.	C_B, Counts in 4 Min.	C_{AB}, Counts in 4 Min.	A, Ag Found, Mg.
53.9	Cl	24.4	6233	1962	53.2
10.8	Cl	24.4	6046	4220	10.5
107.9	Br	20.4	6902	1080	109.9
10.3	I	24.4	5230	1006	10.2
131.5	PO$_4$	28.2	8256	1476	130.0

The accuracy obtained is again of the order of ±2%, as would be expected from the probable errors in the number of counts recorded. The measurements can be carried out easily. The precipitate is added directly to, or suspended in a known volume of, the solution containing the silver nitrate with a known counting rate. After shaking, the final counting rate is found and corrected for the volume change, if any occurred, to the original volume of the radioactive solution.

The method is valuable when the isolation of the pure precipitates is hard to accomplish—for example, in the determination of the total amount of silver in an unknown mixture of silver chloride, bromide, and iodide—without the necessity of isolating a particular halide or electroplating the total silver. Conversely, it may be used for determining any other element in heterogeneous mixtures when the exchange occurs only with the desired precipitate.

LITERATURE CITED

(1) Kolthoff, I. M., *Proc. Acad. Sci. Amsterdam*, **40**, 82 (1937).
(2) Langer, A., *J. Chem. Phys.*, **10**, 321 (1942).
(3) Langer, A., *J. Phys. Chem.*, **45**, 639 (1941).
(4) Langer, A., and Stevenson, D. P., Ind. Eng. Chem., Anal. Ed., **14**, 770 (1942).
(5) Moeller, J., and Schweitzer, G. K., Anal. Chem., **20**, 1201 (1948).
(6) Polessitsky, A., *Compt. rend. acad. sci. U.R.S.S.*, **26**, 668 (1939).

Reactions of Activated Glycerol Dichlorohydrin with Vitamin A

R. S. ALLEN AND SIDNEY W. FOX

Iowa State College, Ames, Iowa

The activation of glycerol dichlorohydrin (GDH) by the addition of small quantities of concentrated hydrochloric acid, concentrated sulfuric acid, or chlorosulfonic acid has been investigated. The absorption spectra of the products of the reaction of vitamin A acetate with these acid-activated reagents are presented. Activation by vacuum distillation of GDH with antimony trichloride produces a more stable reagent, and the color after reaction with vitamin A is more stable than that produced by any of the acid-activated reagents. Compounds that react readily with hydrogen chloride appear to prevent the normal GDH–vitamin A color reaction. Absorption spectra of the reactions of activated GDH with vitamin A, when solvent-reagent ratios of 1:9, 1:5, 1:1, 5:1, and 9:1 were employed, are presented.

THE use of glycerol dichlorohydrin (GDH) as a colorimetric reagent for the measurement of vitamin A was reported in 1945 by Feinstein (5) and Sobel and Werbin (16). Later it was shown (15) that some batches of GDH gave no color with vitamin A but that the reagent could be activated by the addition of concentrated hydrochloric acid, concentrated sulfuric acid, acetyl chloride, benzoyl chloride, phosphorus pentachloride, anhydrous aluminum chloride, stannic chloride, or zinc chloride. Activation was also accomplished by codistillation of GDH with 1 to 5% of antimony trichloride at 4 to 40 mm. pressure; this reagent was recommended for analytical purposes (13–15). Such material has been used for the determination of vitamin A in dairy-calf blood plasma (1; 8, 20), cow blood plasma (17, 18), rat blood serum (12), rat liver and kidney (7), human blood serum (13), fish liver oils (2, 3, 14), cows' milk (18), human milk (11), and fortified poultry mashes (19).

Increasing recognition of the utility of this analytical method made it desirable to study systematically factors which influence the genesis, extent, and nature of the chromogenic response.

Penketh (9) suggested that the activating principle is hydrochloric acid (small quantities of which are formed during distillation with antimony trichloride) or perhaps hydrogen ions since sulfuric acid has some activating effect. Penketh found that activation by addition of about 2% of concentrated hydrochloric acid produced a reagent which, if used within a short time, behaved in a similar manner to reagent activated in the usual manner. On standing, the activation increased somewhat but the desirable stability of the chromophore was lost.

Sobel and Snow (13) reported that no treatment of reagent grade chemicals was necessary for the determinations of carotene and vitamin A in human serum. Special drying treatments appeared to be unnecessary. No other indications regarding the effect of impurities in the reagents seem to have been reported. However, the presence in some biological materials of substances which suppress the GDH–vitamin A color reaction has been recognized (1, 19), although the chemical nature of these color inhibitors has not been elucidated.

Sobel and Werbin (15) also originally advocated the use of a 1:4 solvent-reagent ratio in analytical work but gave no reason for choice of that reaction condition. Chilcote, Guerrant, and Ellenberger (3) found that essentially the same color was produced by 1:4 and 1:5 ratios of solvent to reagent.

In order to achieve a better understanding of the chemistry of GDH as a vitamin A assay reagent, the present study was designed for the investigation of three aspects of the problem:

1. A comparison of the antimony trichloride activation technique with the method involving the addition of acids to inactive GDH.
2. The effects of the addition of known compounds to GDH in order to determine which types of compounds suppress the color reaction.
3. The effect of various solvent to reagent ratios on the absorption spectra of the products of the reactions of vitamin A with activated GDH.

EXPERIMENTAL

Materials. Standard vitamin A solutions were prepared by dissolving weighed quantities of U.S.P. Vitamin A Reference Standard Oil (crystalline vitamin A acetate dissolved in cottonseed oil) in redistilled U.S.P. grade chloroform (dried over anhydrous sodium sulfate). These solutions were used within a few hours after preparation.
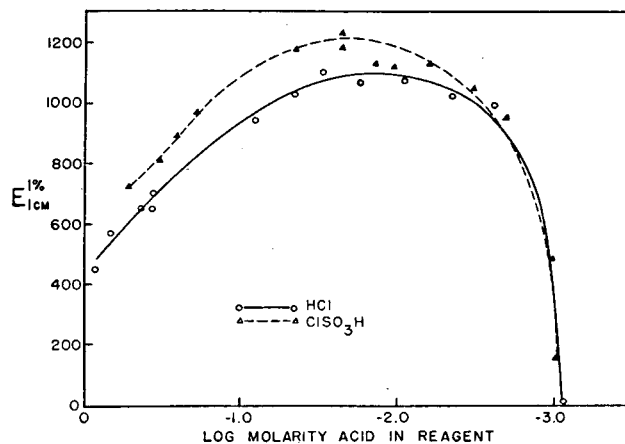

Figure 1. Activation of GDH with Anhydrous Hydrogen Chloride and with Chlorosulfonic Acid

Glycerol (75% 2,3-; 25% 1,3-) dichlorohydrin obtained from Shell Chemical Company was used in the activation studies. After two or more vacuum distillations at 14 to 20 mm. pressure, this GDH was obtained in a clear, colorless, and inactive state.

The activated GDH used in the study of the effect of certain compounds and of the solvent to reagent ratios on the GDH–vitamin A color was purchased from J. B. Shohan Laboratories,

Newark, N. J. This reagent was used without further treatment.

Activation Methods. Activation with acids was accomplished by the addition to GDH of known quantities of concentrated hydrochloric acid (c.p.), concentrated sulfuric acid (c.p.), and chlorosulfonic acid (Eastman, practical grade), and also by passing dry, freshly generated hydrogen chloride directly into inactive GDH. The molarity of hydrogen chloride in these reagents was determined by titration with standard sodium hydroxide using phenolphthalein as the indicator. The formation of a pink color which remained for about 5 seconds was designated the end point since it was found that GDH would itself react slowly with aqueous sodium hydroxide. The molarity of hydrogen chloride in some of the lots of GDH was checked by weighing before and after the gas had been introduced. These solutions were diluted with inactive GDH to give reagents having several concentrations of activating agent.

A batch of GDH was activated by adding 1.06% of antimony trichloride and subjecting the mixture to vacuum distillation according to the method prescribed by Sobel and Snow (*13*).

Apparatus. The absorption spectra of the products of the reactions of vitamin A with activated GDH were obtained with the aid of a Cary recording spectrophotometer, Model 12 (manufactured by the Allied Physics Corporation, Pasadena, Calif.) Accurately measured quantities of reactants were thoroughly mixed in 50-ml. glass-stoppered centrifuge tubes and then poured into the 50-mm. light–path absorption cells. The cells were placed in the instrument within 1 minute and spectral absorption tracings were begun 1.50 minutes (±2 seconds) after the reactions were initiated. The reactions were carried out at 26° ± 1° C. Tracings were begun at a wave length of 700 mμ and proceeded in the direction of shorter wave lengths at a scanning speed of 2 mμ per second. At the conclusion of a tracing, the scanning and chart motors were stopped, and the machine was adjusted to the original starting position on the tracing chart. Another curve was then traced over the same spectral range. This procedure was repeated until seven or eight tracings had been made. Each series of curves, which actually consisted of plots of optical density against wave length, thus indicated the change in the absorption spectra as the reaction mixtures aged.
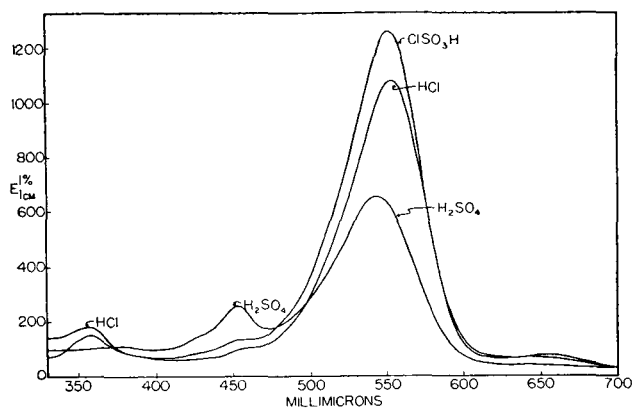

Figure 2. Absorption Spectra of Colors Produced by Reactions of Vitamin A with GDH

Activated with concentrated hydrochloric acid, concentrated sulfuric acid, and chlorosulfonic acid

The Beckman quartz spectrophotometer, Model DU, was employed in the measurements at 555 mμ of the optical densities of several reaction mixtures.

Calculations. All optical density values obtained with the Beckman and Cary spectrophotometers were converted to the corresponding extinction coefficients ($E_{1\,cm.}^{1\%}$) by means of the Bouguer-Beer law:

$$E_{1\,cm.}^{1\,\%} = \frac{D}{c \times l} \tag{1}$$

where D is the observed optical density, c is the number of grams of chromogen per 100 ml. of solution, and l is the thickness of the absorption cell in centimeters.

Table I. Stability of Colors Resulting from Reaction of Vitamin A with Acid-Activated GDH

Acid Concn. in GDH, M	$E_{1\,cm.}^{1\%}$ at Various Times, Min.					
	5	10	15	20	25	30
HCl			553 mμ			
0.012	1012	808	615	470	353	279
0.06	892	645	440	310	225	173
0.12	840	574	382	263	192	150
0.24	787	517	330	220	162	135
0.48	652	370	212	148	118	109
ClSO₃H						
0.0096	1214	1070	913	778	655	543
0.054	1114	945	788	656	544	455
0.106	1047	932	808	700	605	528
H₂SO₄			545 mμ			
0.018	588	628	614	589	563	536
0.09	520	540	499	462	432	402
0.18	636	688	682	655	630	607

RESULTS AND DISCUSSION

Acid Concentration for Optimum Activation. The concentration of hydrogen chloride or chlorosulfonic acid necessary to give maximum activation of GDH was determined by mixing 1.0 ml. of vitamin A solution with 4.0 ml. of reagent and measuring the optical density in the Beckman spectrophotometer at 555 mμ, 2 minutes after initiation of the reaction. The 2-minute extinction coefficients were plotted against the logarithm of the molarity of acid in the reagent (Figure 1). Optimum activation was obtained when the reagent was approximately 0.01 M with respect to hydrogen chloride or 0.02 M in chlorosulfonic acid. Relatively high activation resulted when the concentration of hydrogen chloride ranged from 0.002 M to 0.08 M and when the chlorosulfonic acid concentration was between 0.004 M and 0.1 M.

After several of these acid-activated reagents had been stored for about 3 weeks in clear, glass-stoppered bottles the activity was rechecked. Reagents containing relatively large quantities of acid were found either to increase in activity or to decrease slightly. Those reagents which had optimum activity, when freshly prepared, were found to decrease in activity, whereas those containing only traces of activating acids and having low initial activity increased in activity on standing in the laboratory. This activity, however, decreased on continued storage.

Inactive GDH became somewhat active on standing for several weeks in a glass-stoppered clear bottle exposed to laboratory light at room temperature. Several brands of GDH gave the same result. The rate of activation by light alone was less when
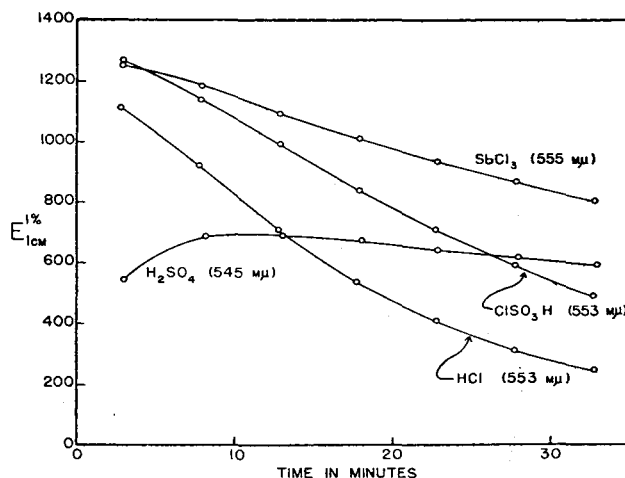

Figure 3. Stability of Colors Produced by Reactions of Vitamin A with GDH

Activated with hydrochloric acid, sulfuric acid, and chlorosulfonic acid, also by vacuum distillation with 1% antimony trichloride

the reagents were stored in brown bottles. This activation appeared to result from decomposition of GDH to give hydrogen chloride as one of the products. Inactive GDH had no free chloride ion when tested with alcoholic silver nitrate, but after activity appeared the chloride ion test was strongly positive.
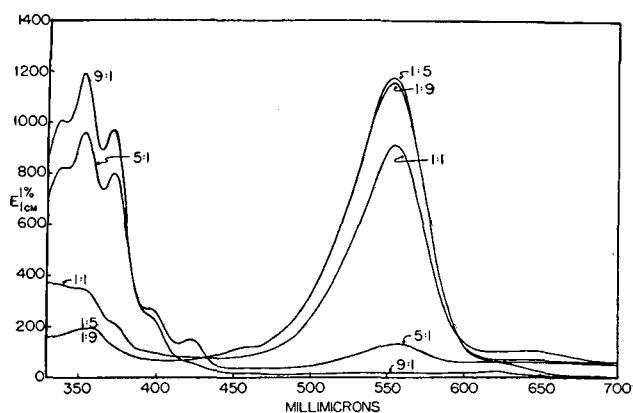

Figure 4. Absorption Spectra of Products of Reactions of Shohan GDH with Vitamin A at Several Solvent-Reagent Ratios

Absorption Spectra of GDH–Vitamin A Reaction Products. Four volumes of each freshly activated GDH were mixed with one volume of standard chloroform solution of vitamin A, and the absorption spectrum of the reaction mixture was determined with the aid of the Cary recording spectrophotometer as indicated. In each reaction the initially formed blue color changed to violet. Figure 2 shows the absorption spectra of the violet colors resulting when reagents activated with concentrated hydrochloric acid (0.012 M), concentrated sulfuric acid (0.18 M), and chlorosulfonic acid (0.0096 M) were employed. The absorption spectrum of the antimony trichloride–activated reagent was the same as that described by Sobel and Werbin (15) and corresponds to that of the reaction involving the hydrochloric acid–activated GDH. The principal absorption maximum produced by the reagents activated by hydrochloric acid and chlorosulfonic acid was at 553 mμ; rather weak bands occurred at 358 and 452 mμ. Sulfuric

acid–activated GDH gave a violet color with absorption maxima at 545 and 452 mμ.

Table I summarizes the extinction coefficients obtained by interpolation from a series of absorption spectra traced by the Cary recording spectrophotometer. The concentrations of acids present in the activated reagents are given in the first column. Activity of reagent and the stability of the color (553 mμ) produced by its reaction with vitamin A increased with decreasing concentrations of either hydrochloric acid or chlorosulfonic acid. Activity of the sulfuric acid–activated reagent and stability of the color (545 mμ) were greatest when the acid concentration was 0.18 M. It was also observed that the absorption at 452 mμ increased with increasing sulfuric acid concentration in GDH.

Figure 3 compares the stability of the colors resulting from the reactions of vitamin A with GDH activated by addition of hydrochloric acid (0.012 M), sulfuric acid (0.18 M), chlorosulfonic acid (0.0096 M), and also by vacuum distillation with 1.06% antimony trichloride. The antimony trichloride–activated GDH produced a more stable color than did either the hydrochloric acid– or chlorosulfonic acid–activated reagents. Of the acid-activated reagents, the chlorosulfonic acid treatment was superior to the hydrochloric acid or sulfuric acid, but for over-all utility as an analytical reagent the antimony trichloride–activated GDH ranks highest.

Effect of Several Compounds on GDH–Vitamin A Color Reaction. Several levels of the following substances were added directly to activated Shohan GDH: water, 95% ethyl alcohol, dioxane, epichlorohydrin, pyridine, n-butylamine, and aniline. Table II summarizes the influence of these impurities on the GDH–vitamin A color extinction coefficient 2 minutes after initiation of the reactions. Epichlorohydrin, pyridine, n-butylamine, and aniline were effective in preventing color formation, whereas water, dioxane, or ethyl alcohol had much less influence on the color development. Aqueous potassium hydroxide was also tested
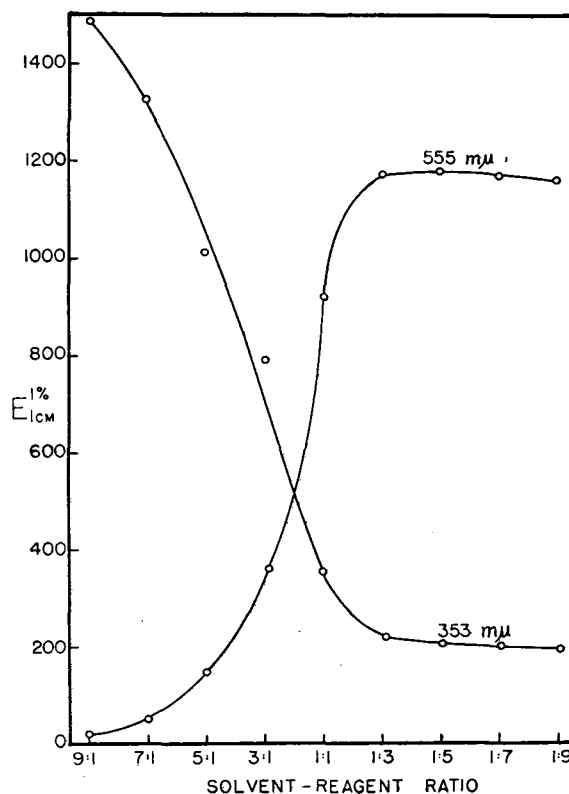
Table II. Effect of Some Compounds on Color Reaction of Shohan GDH with Vitamin A

Compd. Added to Reagent, M	$E_{1 cm.}^{1\%}$ at 555 mμ^a	Compd. Added to Reagent, M	$E_{1 cm.}^{1\%}$ at 555 mμ^a
Pyridine		Dioxane	
0.0000	1320	0.0000	1294
0.0011	1142	0.0313	878
0.0022	1048	0.0626	693
0.0055	0	0.157	597
0.0111	0	0.3130	272
n-Butylamine		95% Ethyl Alcohol	
0.0000	1310	0.0000	1256
0.0017	705	0.0396	1218
0.0034	309	0.0792	1195
0.0085	29	0.198	991
0.0169	25	0.396	836
Aniline		Water	
0.0000	1310	0.000	1424
0.0022	1009	0.113	1115
0.0045	445	0.227	947
0.0112	41	0.567	586
0.0224	0	1.134	71
Epichlorohydrin			
0.0000	1260		
0.0026	1219		
0.0524	1094		
0.0131	10		
0.0262	0		

^{a} All values based on optical density readings 2 minutes after mixing a chloroform solution of vitamin A with the reagents.

Figure 5. Influence of Solvent-Reagent Ratio on Extinction Coefficients at 353 and 555 mμ

Reactions of vitamin A with Shohan GDH

and was found to suppress the color formation more than water, ethyl alcohol, or dioxane.

It appears that compounds having an affinity for hydrogen chloride cause inhibition of the GDH–vitamin A color reaction. The reason for this must lie in the reaction with free hydrogen chloride which is probably the main activating principle in the antimony trichloride–activated GDH. In addition there may be a possibility of reaction of the amines with GDH to form amino derivatives followed by the formation of the hydrochlorides. Dioxane, water, and alcohol could possibly cause some inhibition by the formation of oxonium salts with hydrogen chloride.

Solvent-Reagent Ratio. Ratios ranging from 1:9 to 9:1 were chosen for study. Activated Shohan GDH was mixed with standard solutions of vitamin A in chloroform and the absorption spectra of the reaction mixtures were determined with the Cary recording spectrophotometer. The initial absorption spectra of the reaction products obtained when the 9:1, 5:1, 1:1, 1:5, and 1:9 solvent-reagent ratios were used are shown in Figure 4. The absorption at 555 mμ decreased while that at 353 mμ increased as the quantity of solvent in the reaction mixture was increased. In addition new absorption maxima appeared at 337, 372, 395, and 422 mμ when high solvent-reagent ratios were used. The same types of spectra were also observed with antimony trichloride–activated Shell, Paragon, and Eastman GDH.
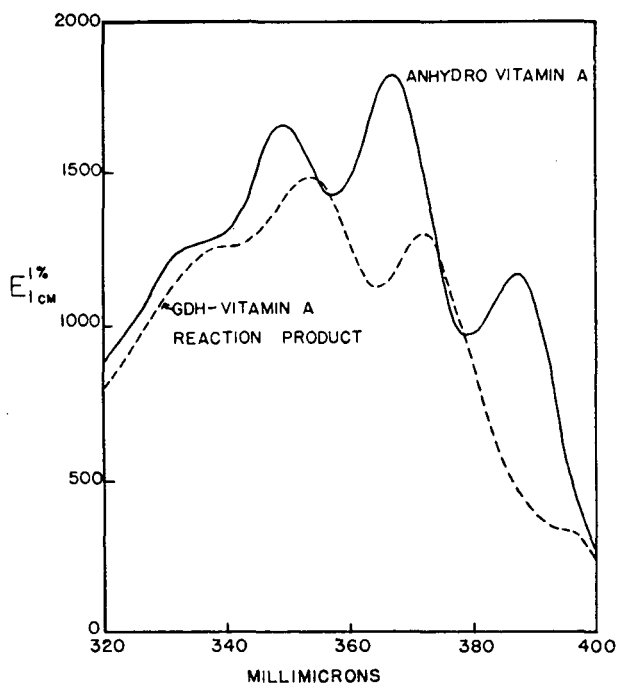

Figure 6. Ultraviolet Absorption Spectra of GDH–Vitamin A Reaction Product

Solvent to reagent ratio. 5:1; product of reaction of vitamin A acetate with 0.033 N hydrochloric acid in ethyl alcohol

Table III indicates the stability of the 555- and 353-mμ maxima when the several solvent-reagent ratios were employed. The extinction coefficients were interpolated from the series of absorption spectra traced by the Cary recording spectrophotometer. The stability and intensity of the 555-mμ maximum were improved by using low solvent-reagent ratios, whereas the use of high ratios increased the extinction coefficient but did not improve the stability at 353 mμ.

Figure 5 shows the influence of the solvent-reagent ratio on the absorption at 555 and 353 mμ. By use of the Cary recording spectrophotometer, the measurements at 555 mμ were made at 2.7 minutes and those at 353 mμ were made 4.4 minutes after initia-

Table III. Effect of Solvent-Reagent Ratio on Stability at 555 and 353 mμ of Product of Vitamin A–Shohan GDH Reaction

Solvent-Reagent Ratio	$E_{1\ cm}^{1\%}$ at Various Times, Min.					
	5	10	15	20	25	30
			555 mμ			
1:9	1110	972	850	733	630	545
1:5	1125	983	843	728	615	525
1:1	835	653	505	385	298	234
5:1	164	137	95	73	62	57
9:1	55	70	56	50	47	43
			353 mμ			
1:9	193	207	216	223	228	237
1:5	210	216	221	230	238	243
1:1	332	294	288	286	286	286
5:1	978	853	752	664	588	528
9:1	1420	1232	1120	1044	983	928

tion of the reactions. The extinction coefficients at 555 and 353 mμ were essentially constant when solvent-reagent ratios of 1:3, 1:5, 1:7, and 1:9 were employed. This is in agreement with the report of Chilcote et al. (3) who indicated that essentially the same color was produced by using either 1:4 or 1:5 ratios of solvent to reagent.

The absorption spectrum of the product resulting from the use of a high solvent-reagent ratio resembles that of anhydro vitamin A. This compound has main absorption maxima at 350, 370, and 392 mμ when prepared by dehydration of vitamin A alcohol with 0.033 N hydrochloric acid in ethyl alcohol (4, 6, 10). Since the form of the vitamin A used in the present investigation was that of the acetate, the dehydration reaction was investigated spectrophotometrically. After mixing 1 ml. of chloroform solution containing vitamin A acetate equivalent to 53 γ of vitamin A alcohol with 6 ml. of 0.033 N hydrochloric acid in ethyl alcohol at room temperature, absorption spectral curves were traced at 6.5-minute intervals over a 90-minute period starting at 400 mμ and proceeding toward shorter wave lengths at a scanning speed of 1 mμ per second. The fine line-type structure developed rather slowly. The first distortion in the vitamin A acetate absorption spectrum was at the 388-mμ band and the last band to appear was at 348 mμ. The time required for maximum absorption followed the same order: 388 mμ, 54 minutes; 367 mμ, 67 minutes; and 348 mμ, 75 minutes. Figure 6 includes the absorption spectrum of this reaction product after the reaction had proceeded for 1 hour and also the absorption spectrum of the product of the reaction of vitamin A acetate with GDH when a 5:1 solvent-reagent ratio was used. The latter is not identical to the former; the principal differences are a shift of the maxima toward longer wave lengths and a progressive decrease in absorption as the wave length maxima approach 400 mμ.

SUMMARY

Quantitative studies indicated that high activity of GDH was produced by concentrations of anhydrous hydrogen chloride ranging from 0.002 M to 0.08 M with optimum activity at 0.01 M hydrogen chloride. High activity was produced by concentrations of chlorosulfonic acid ranging from 0.008 M to 0.1 M with optimum activity at 0.02 M chlorosulfonic acid.

Activation of GDH by vacuum distillation with 1% antimony trichloride produced a reagent with high activity and the color after reaction with vitamin A was more stable than that produced by the acid-activated reagents. In addition, aging at room temperature resulted in changes in activity of the reagents activated with either hydrochloric acid or chlorosulfonic acid. Because of the lack of reagent stability and also GDH–vitamin A color stability, the acid-activation methods are inferior in the preparation of analytical reagents.

Pyridine, aniline, n-butylamine, and epichlorohydrin inhibit the GDH–vitamin A color reaction. Aqueous potassium hydroxide, ethyl alcohol, water, and dioxane had some inhibitory action.

Apparently compounds that react readily with hydrogen chloride prevent the normal color reaction.

Absorption spectra of the reactions of activated GDH with vitamin A when solvent to reagent ratios of 1:9, 1:5, 1:1, 5:1, and 9:1 were employed are presented. The extinction coefficients at 555 mμ were essentially the same in the cases of the 1:9, 1:7, 1:5, and 1:3 ratios. As the ratios were increased above 1:3, the absorption at 555 mμ decreased while the absorption at 337, 353, 372, 395, and 422 mμ increased and reached maximum values when a 9:1 ratio was used. These maxima in and near the ultraviolet resemble to some extent the type absorption spectrum of anhydro vitamin A.

ACKNOWLEDGMENT

The kindness of F. H. Spedding in making the Cary recording spectrophotometer available is acknowledged.

LITERATURE CITED

(1) Allen, R. S., Wise, G. H., and Jacobson, N. L., *J. Dairy Sci.*, 32, 688 (1949).
(2) Braekkan, O. R., ANAL. CHEM., 21, 1530 (1949).
(3) Chilcote, M. E., Guerrant, N. B., and Ellenberger, H. A., *Ibid.*, 21, 1180 (1949).
(4) Edisbury, J. R., Gillam, A. E., Heilbron, I. M., and Morton. R. A., *Biochem. J.*, 26, 1164 (1932).
(5) Feinstein, L., *J. Biol. Chem.*, 159, 569 (1945).
(6) Hanze, A. R., Conger, T. W., Wise, E. C., and Weisblat, D. I., *J. Am. Chem. Soc.*, 70, 1253 (1948).
(7) Kelley, B., and Day, H. G., *J. Nutrition*, 40, 159 (1950).
(8) Murley, W. R., Jacobson, N. L., Wise, G. H., and Allen, R. S., *J. Dairy Sci.*, 32, 609 (1949).
(9) Penketh, D. D., *Nature*, 161, 893 (1948).
(10) Shantz, E. M., Cawley, J. D., and Embree, N. D., *J. Am. Chem. Soc.*, 65, 901 (1943).
(11) Sobel, A. E., and Rosenberg, A. A., ANAL. CHEM., 21, 1540 (1949).
(12) Sobel, A. E., Sherman, M., Lichtblau, J., Snow, S., and Kramer, B., *J. Nutrition*, 35, 225 (1948).
(13) Sobel, A. E., and Snow, S. D., *J. Biol. Chem.*, 171, 617 (1947).
(14) Sobel, A. E., and Werbin, H., ANAL. CHEM., 19, 107 (1947).
(15) Sobel, A. E., and Werbin, H., IND. ENG. CHEM., ANAL. ED., 18, 570 (1946).
(16) Sobel, A. E., and Werbin, H., *J. Biol. Chem.*, 159, 681 (1945).
(17) Squibb, R. L., Cannon, C. Y., and Allen, R. S., *J. Dairy Sci.*, 31, 421 (1948).
(18) *Ibid.*, 32, 565 (1949).
(19) Wall, M. E., and Kelley, E. G., ANAL. CHEM., 20, 757 (1948).
(20) Yang, S. P., M.S. thesis, Iowa State College, 1949.

Quantitative Analysis of Mixtures of Primary, Secondary, and Tertiary Aromatic Amines

SIDNEY SIGGIA, J. GORDON HANNA, AND IRENE R. KERVENSKI

General Aniline & Film Corporation, Easton, Pa.

A method has been devised for determining primary, secondary, and tertiary aromatic amines in the presence of each other. The final analyses are acidimetric in nature; however, the sample is first altered in various ways to make the determination of each component possible.

THE quantitative analysis of mixtures of amines has been a problem for many years. Wagner, Brown, and Peters (5) devised a system for determination of primary, secondary, and tertiary aliphatic amines, but this system could not be applied to aromatic mixtures because of the much weaker basic properties of the aromatic amines. The methods described below utilize the same reactions as those devised by Wagner, Brown, and Peters, but the reaction media and techniques used make possible the utilization of the analysis system for the determination of aromatic amine mixtures. This method is also readily applicable to aliphatic amine systems, but the aliphatic systems are adequately covered by Wagner, Brown, and Peters.

Mitchell, Hawkins, and Smith (2) proposed determining tertiary amines by determining first the sum of primary and secondary amines by acetylation, measuring the excess anhydride by aquametric means; the tertiary amine was obtained by determining total base and subtracting the sum of primary and secondary amines. In aromatic systems the amines involved are so weakly basic that titration of total base for determination of tertiary amines is impossible by ordinary means. Also, the acetylation procedure used for determining primary plus secondary amines cannot be used when alcohols are present with the amines. The procedure has an unnecessary step in the addition of excess water and determination of the water by the Karl Fischer reaction. Simply determining the excess anhydride after acetylation by titration with sodium hydroxide has been found to yield very good results.

Hawkins, Smith, and Mitchell (1) also devised a procedure for determining primary amines in the presence of secondary and tertiary amines. The sample is reacted with benzaldehyde and the water liberated is determined by the Karl Fischer reaction. This procedure involves the use of hydrogen cyanide, which necessitates special handling and is more time-consuming than the method described below.

In the method for determining aromatic amine mixtures which is described in this paper the tertiary amine is determined by adding acetic anhydride directly to a weighed sample. After a short time the acetylated mixture is dissolved in 1 to 1 ethylene glycol–isopropyl alcohol, and the tertiary amine (which is not affected by the anhydride) can be titrated using standard hydrochloric acid. The glycol–isopropyl alcohol solvent is used to accentuate the titration of the tertiary amine, which is a weak base. It was first proposed by Palit (4) to make possible the titration of weak bases that could not be accurately determined by titration in aqueous solution.

The primary aromatic amine in the mixture is determined by titrating the total base in the sample in the 1 to 1 ethylene glycol-isopropyl alcohol solvent. To a separate sample salicylaldehyde is added to remove the primary amine via the Schiff reaction, and the remaining base in the sample is then titrated. The difference between the two titrations will yield the primary amine content.

The secondary amine content is determined by taking the titration value after addition of salicylaldehyde: tertiary amine

plus secondary amine. By subtracting the value obtained for the tertiary amine as described above, the amount of secondary amine can be determined.

REAGENTS

Ethylene glycol–isopropyl alcohol mixture, 1 to 1.

Standard 1 N hydrochloric acid in ethylene glycol–isopropyl alcohol mixture, 96 ml. of concentrated hydrochloric acid diluted to 1 liter with 1 to 1 ethylene glycol–isopropyl alcohol.

c.p. acetic anhydride.

Salicylaldehyde (from bisulfite addition compound).

PROCEDURES

Procedure A. Total Amines. A sample containing approximately 0.02 mole of total amines is accurately weighed in a weighing bottle. The contents of the weighing bottle are washed into a 150-ml. beaker with 1 to 1 ethylene glycol–isopropyl alcohol mixture and ethylene glycol–isopropyl alcohol mixture is added until the volume is approximately 50 ml. A pH meter is used to indicate the apparent pH after each addition of acid, as the sample is titrated with 1 N hydrochloric acid prepared in the ethylene glycol–isopropyl alcohol mixture. The neutralization point is determined by plotting the apparent pH against milliliters of acid.

$$\frac{\text{Ml. of HCl} \times N}{\text{grams of sample} \times 1000} =$$

moles of total amines per gram of sample

Procedure B. Secondary plus Tertiary Amines. A sample containing approximately 0.02 mole total of secondary and tertiary amines is accurately weighed in a weighing bottle. The contents of the weighing bottle are washed into a 150-ml. beaker with 1 to 1 ethylene glycol–isopropyl alcohol mixture, and ethylene glycol–isopropyl alcohol mixture is added until the volume is approximately 50 ml. Five milliliters of salicylaldehyde are added (more if the amount of primary amine is larger than 0.035 mole). The mixture is stirred thoroughly and allowed to stand at room temperature for 0.5 hour. A pH meter is used to indicate the apparent pH after each addition of acid as the sample is titrated with 1 N hydrochloric acid prepared in the ethylene glycol–isopropyl alcohol mixture. The neutralization point is determined by plotting apparent pH against milliliters of acid.

$$\frac{\text{Ml. of HCl} \times N}{\text{grams of sample} \times 1000} =$$

moles of secondary plus tertiary amine per gram of sample

Procedure C. Tertiary Amines. A sample which contains approximately 0.02 mole of tertiary amine is accurately weighed in a 20 $\times$ 150 mm. test tube and cooled by placing in a beaker of ice. Ten milliliters of acetic anhydride are added slowly while the test tube is swirled. The test tube and contents are allowed to stand 15 minutes at room temperature. The contents are quantitatively transferred from the test tube into a 150-ml. beaker by washing with 1 to 1 ethylene glycol–isopropyl alcohol mixture.

Ethylene glycol–isopropyl alcohol mixture is added until the volume is approximately 50 ml. A pH meter is used to indicate the apparent pH as the sample is titrated with 1 N hydrochloric acid prepared in the ethylene glycol–isopropyl alcohol mixture. The neutralization point is determined by plotting the apparent pH against milliliters of acid.

Table I. Determination of Primary, Secondary, and Tertiary Amines

System	Primary, % Calcd.	Primary, % Found	Secondary, % Calcd.	Secondary, % Found	Tertiary, % Calcd.	Tertiary, % Found
Aniline, ethylaniline, and diethylaniline	33.29	33.05	31.89	31.87	34.79	34.90
	74.95	75.41	12.02	11.29	13.02	12.96
	9.70	10.52	43.30	42.04	47.00	47.32
Aniline, methylaniline, and dimethylaniline	33.39	32.86	33.34	32.61	33.24	34.00
	74.97	74.57	12.50	12.44	12.58	12.52
	9.77	10.43	45.13	45.27	45.05	45.42
1-Naphthylamine, ethyl-1-naphthylamine, and diethyl-1-naphthylamine	34.16	33.34	32.96	33.78	32.88	32.50
	9.89	10.14	45.07	44.53	45.04	44.74
1-Naphthylamine, and dimethyl-1-naphthylamine	49.96	49.38	a	...	46.44	46.59
	18.17	18.92	a	...	75.94	76.21

a Secondary amines of this system could not be obtained.

$$\frac{\text{Ml. of HCl} \times N}{\text{grams of sample} \times 1000} =$$

moles of tertiary amines per gram of sample

Calculations. PRIMARY AMINE

$$\frac{\text{Moles of total amine}}{\text{gram}} - \frac{\text{moles of sec.} + \text{tert. amine}}{\text{gram}} = \frac{\text{moles of primary amine}}{\text{gram}}$$

$$\frac{\text{Moles of primary amine}}{\text{gram}} \times \text{mol. wt. of primary amine} \times 100 = \% \text{ primary amine}$$

SECONDARY AMINE

$$\frac{\text{Moles of sec. amine} + \text{tert. amine}}{\text{gram}} - \frac{\text{moles of tert. amine}}{\text{gram}} = \frac{\text{moles of sec. amine}}{\text{gram}}$$

$$\frac{\text{Moles of sec. amine}}{\text{gram}} \times \text{mol. wt. of sec. amine} \times 100 = \% \text{ sec. amine}$$

TERTIARY AMINE

$$\frac{\text{Moles of tert. amine}}{\text{gram}} \times \text{mol. wt. of tert. amine} \times 100 = \% \text{ tert. amine}$$

DISCUSSION

One of the desirable features of this system of analysis is the small number of interferences. First of all, an interference has to be basic. In the determination of tertiary amines, any basic impurity is neutralized by the acetic anhydride and is thus removed. In the primary amine determination, it is the decrease in basicity on addition of salicylaldehyde that is measured. Any basic impurity is figured in both titrations and does not affect the difference.

A basic impurity will affect only the determination of the secondary amine. However, if the alkaline impurity is strong enough, it can be determined by a differential titration in the presence of the aromatic amines, which are very weak bases. The secondary amine value can then be corrected. Ammonia present in mixtures of aniline and monoethyl and diethylaniline was handled very satisfactorily by the above technique.

It was found impossible to determine N,N-di-(β-hydroxyethyl)-aniline by the tertiary amine procedure. On acetylation, the hydroxyl groups on the molecule were esterified, and this resulted in such a decrease in the basicity of the compound that it was no longer titratable even in the special solvent mixture. Systems containing diphenylamine and triphenylamine could not be determined because these materials are too weakly basic to be titrated.

In the determination of secondary plus tertiary amines, the buffering action of the Schiff base formed after the reaction with salicylaldehyde is sometimes strong enough to decrease the sensitivity of the titration curve. In these cases, it is advisable to make the plotted curve more sensitive by extending the pH scale of the graph over a longer length, so that each division on the graph equals a smaller pH unit. In this way, the break in the curve is accentuated, and a more accurate reading is made possible.

The above procedures provide an easily applicable way of determining primary, secondary, and tertiary amines when all are present in the same sample. Combinations of the above procedures can also be applied to determine mixtures when only two of the three types of amines are present. However, in the latter case, other techniques can sometimes be used which give the analysis for the two components more directly, more accurately, or faster.

The procedure for tertiary amines described above is the most efficient for these compounds in the presence of primary or secondary amines.

The procedure for primary amines as described above is the only known method for determining these compounds in the presence of secondary amines. However, if the mixture to be analyzed consists of a primary and tertiary amine, the primary amine is best determined by the acetylation procedure of Ogg, Porter, and Willits (*3*), which is faster than the salicylaldehyde method. Alcohols interfere with the acetylation method.

The acetylation method is also the best way of determining secondary amines in the presence of tertiary amines; it is more direct than determining total amines and subtracting the tertiary amine.

LITERATURE CITED

(1) Hawkins, W., Smith, D. M., and Mitchell, J., Jr., *J. Am. Chem. Soc.*, **66**, 1062 (1944).
(2) Mitchell, J., Jr., Hawkins, W., and Smith, D. M., *Ibid.*, **66**, 782 (1944).
(3) Ogg, C. L., Porter, W. L., and Willits, C. O., IND. ENG. CHEM., ANAL. ED., **17**, 397 (1945).
(4) Palit, S., *Ibid.*, **18**, 246–51 (1946).
(5) Wagner, C. D., Brown, R. H., and Peters, E. D., *J. Am. Chem. Soc.*, **69**, 2609–14 (1947).

Direct Determination of Oxygen in Compounds Containing Carbon, Hydrogen, and Oxygen

A Physical-Chemical Technique

CHARLES C. HARRIS, DONALD M. SMITH, AND JOHN MITCHELL, JR.
Polychemicals Department, E. I. du Pont de Nemours & Company, Inc., Wilmington, Del.

A new technique is presented for the direct determination of oxygen in organic compounds, based on the Unterzaucher carbon-reduction procedure. The sample is decomposed in a stream of helium and the pyrolysis products are circulated in a closed system over carbon at 1100° to 1150° C. until conversion of all the oxygen to carbon monoxide is complete. During circulation, essentially all the hydrogen is removed selectively by diffusion through a heated palladium tube. This procedure permits the use of very large samples, which is of particular value in the analysis of materials of low oxygen content. Using a thermal conductivity bridge, the composition of the resultant helium–carbon monoxide mixture is determined with a sensitivity of 0.20% carbon monoxide per mv. The amount of carbon monoxide formed from the oxygen in the sample may be calculated after measurement of the total volume of gas. Because the thermal conductivity measurements are referred to a control cell, tank helium may be used without further purification. By employing continuous recording of bridge potential, a valuable means is provided for following the course of pyrolysis and conversion. Preliminary results on a wide variety of solid and liquid compounds indicate a relative accuracy of 1% or better.

A TRULY general procedure is not yet available for the direct determination of oxygen in organic compounds. A survey of the methods in the literature (*1, 2, 4, 7, 10, 12*) leads to the conclusion that the technique of Unterzaucher (*12*) is the one most likely to provide the basis for such a procedure.

Unterzaucher's technique was based on the decomposition of the sample in a carrier of nitrogen gas and conversion of the products to carbon monoxide and hydrogen by passage over amorphous carbon at 1100° C. The carbon monoxide was determined by reaction with iodine pentoxide followed by titration of the liberated iodine. The authors' experience with this method paralleled that of Aluise, Hall, Staats, and Becker (*1*) in that high variable blanks and irregular behavior for a large number of compounds were found. The excellent paper published by these authors in 1947 described a procedure in which many of the objections to Unterzaucher's original method (*12*) were overcome. Recently Dinerstein and Klipp (*3*) showed that the technique of Aluise and his co-workers could be extended to the analysis of compounds containing as little as 0.15% oxygen. At these low oxygen contents, the blank may be equivalent to nearly 50% of the total oxygen found. The magnitude of this blank thus may be considered to set the lower limit of the method.

Walton, McCulloch, and Smith (*13*) described a sensitive method for oxygen contents in the range of 0.01 to 6%. Their technique, which also was based on reduction over carbon, employed helium as the carrier gas and used a colorimetric gel for measurement of carbon monoxide. Commenting on the results of unpublished work at the National Bureau of Standards, Walton, McCulloch, and Smith suggested that the blanks previously found in applications of the Unterzaucher technique could be attributed to the iodine pentoxide reagent. These authors stated that in their method the blank was reduced to an amount equivalent to 0.001% oxygen in a sample. In reporting their results, Walton and co-workers indicated a relative accuracy of 2 to 5%.

In the technique described in the present paper, the sample is pyrolyzed in a stream of helium, and the decomposition products are circulated in a closed system over carbon at 1100° C. During circulation, hydrogen is removed selectively by diffusion through a heated palladium membrane, and the rate of formation of carbon monoxide is followed by recording the change in thermal conductivity of the gas mixture. When conversion and hydrogen removal are complete, as indicated by no further change in bridge potential, the total volume of gas is measured

and the amount of carbon monoxide is calculated. This technique is universally applicable to gases, liquids, and solids. At the same time, it appears to be suitable for analyses over the whole range of oxygen contents from 0.1 to 100% with an average relative accuracy of 1%.

APPARATUS

The circulatory unit, which is constructed mainly of borosilicate glass, is shown schematically in Figure 1. It is attached to a high-vacuum system which includes a McLeod gage, a cold trap, and a two-stage mercury diffusion pump backed by a Welch mechanical pump. All stopcocks are high vacuum grade and customarily are lubricated with Apiezon N grease. An ultimate vacuum of less than 10^{-5} mm. of mercury can be readily achieved.

Evacuation of the circulatory unit may be effected through stopcock $SC1$ or through stopcocks $SC3$ and $SC4$, which connect to the vacuum system. $SC5$ is used to admit helium or nitrogen as required. In any single cycle the gas in the unit is circulated by the pump, BC, through the throttling stopcock, $SC2$, the rotameter, D, and the cell, X, of the thermal conductivity bridge, past the stoppered ground joint, H, over the carbon in the quartz converter tube, K, through the glass wool in the dust trap, N, and finally through the hydrogen diffuser, A.
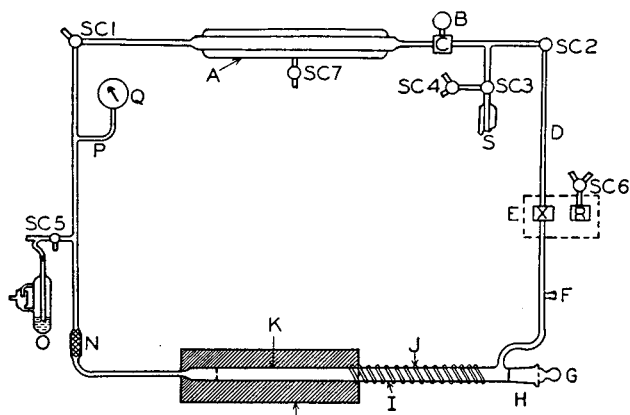

Figure 1.   Circulatory Unit

The positive displacement pump, BC, is variable in capacity from about 20 to 2000 ml. per minute. It is constructed of a silver-plated bellows, B, which opens through a Kovar-to-borosilicate glass graded seal to the glass valve system, C. A Bodine stirring motor is geared to an eccentric drive, which alternately compresses and releases the bellows, creating the necessary pumping action. The valve system, C, consists of two hollow, spherically shaped, glass poppets with tails for guides; these move in a section of 2-mm. capillary tubing below the valve seats. Both poppets and seats are ground and polished. During circulation, the pumping rate is normally set above that required and the flow rate is controlled by the action of the throttling stopcock $SC2$. This reduces surging and maintains an approximately uniform rate of flow. The compound Bourdon gage, Q, indicates the pressure in the unit with sufficient accuracy. It is attached through a Kovar-to-borosilicate glass graded seal, P.

Solid samples are introduced into the pyrolysis section, I, through H (Figure 2). This grind was made in such a way that the side tubulation enters this section at the end of the closed hollow plug, G. G and H were precision ground to a close fit. The grind has a taper of about 15°, is 10 cm. long, and has approximately a 20-mm. inside diameter at the small end. Only the 3-cm. portion at the large end of the grind is lubricated.

Connection of H to the quartz pyrolysis section, I, is made through a borosilicate glass-to-quartz graded seal, $M1$.

Figure 2, which is drawn to scale, shows the detailed design of the section adjacent to the quartz converter. The pyrolysis section, I, is heated by a Nichrome element, J, wrapped directly on the tube and controlled by a Powerstat. The element has a resistance of about 12 ohms when drawing 8 amperes and is capable of heating the sample boat to 550° C. The heating element, J, extends nearly to the grind, H, and acts as a preheater. When temperatures higher than 500° C. are needed, as in the pyrolysis of carbohydrates and polymers, the section containing the sample boat is raised to a temperature of about 800° C. by surrounding it with a radiation shield made of polished stainless steel covered with insulation.

A Kanthal furnace is used to maintain the quartz converter at 1100° to 1150° C. The converter is so positioned in the furnace that the carbon bed ends at the hottest region. In packing the reactor, quartz wool is placed next to the disk orifice. Then carbon pellets are packed into the reactor and held in place with platinum gauze. On the exit side of the reactor is a quartz-to-borosilicate glass graded seal, $M2$. The disk orifice has a 1-mm. opening.

Figure 3 shows in detail the design of the hydrogen diffuser, A. The palladium tube is maintained at a temperature of 450° C. by a Nichrome element wound on a loose quartz sleeve and connected to a Tag controller which is activated by the thermocouple to supply either a high or a low heat. The exit side of the membrane is connected to the high vacuum system through a stopcock ($SC7$). The capacity of the diffuser at 450° C. reduces the partial pressure of hydrogen from about 260 mm. of mercury to less than 1 mm. of mercury in 15 minutes; this amounts to about 250 ml. of hydrogen at standard temperature and pressure.

The barometric pipet shown in Figure 4 contains three standard volumes which were calibrated with mercury. The aggregate volume, totaling about 200 ml., is used in determining the total volume of gas in the unit at the end of a run, whereas the component volumes are used in making up known mixtures of gases for thermal conductivity calibrations. The pipet may also be used in introducing gaseous samples for analysis; it encloses a known volume when the mercury meniscus just touches the tip of any one of the glass pointers. A temperature of 35° ± 0.1° C. is maintained around the pipet by circulating thermostated water through the jacket.

Volatile liquid samples are admitted to the circulatory unit through the horizontal ground joint, F (Figure 1), by means of the device shown in Figure 5. The device consists of sample tube 1 and tube holder 2. The sample tube is provided with a sealed-in sintered glass disk, 3, of medium porosity. The upper end of the sample tube is sealed to an inner 10/20 grind, 4. The sintered disk is covered with about 1 cm. of mercury. Construction of the tube holder is such that, when joined, the holder and the sample tube form a T with the pivotal grind, 5, which is attached to the circulatory unit.
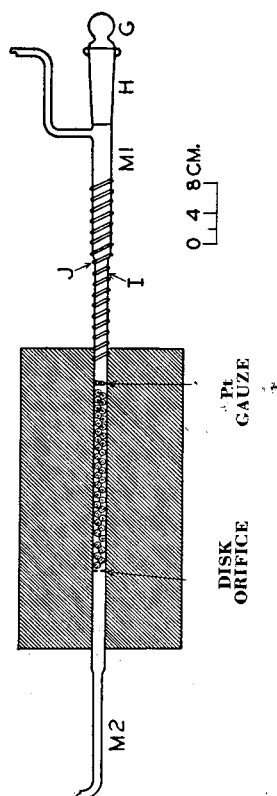

Figure 2.   Converter and Pyrolysis Section

THERMOCOUPLE
WELL (PYREX)

KOVAR-TO-PYREX
GRADED SEAL

PYREX-TO-QUARTZ
GRADED SEAL

KOVAR

NICHROME ELEMENT

QUARTZ SLEEVE

QUARTZ

TO VACUUM

PALLADIUM TUBE
(THICKNESS=0.2MM.)

STAINLESS BELLOWS

TUNGSTEN
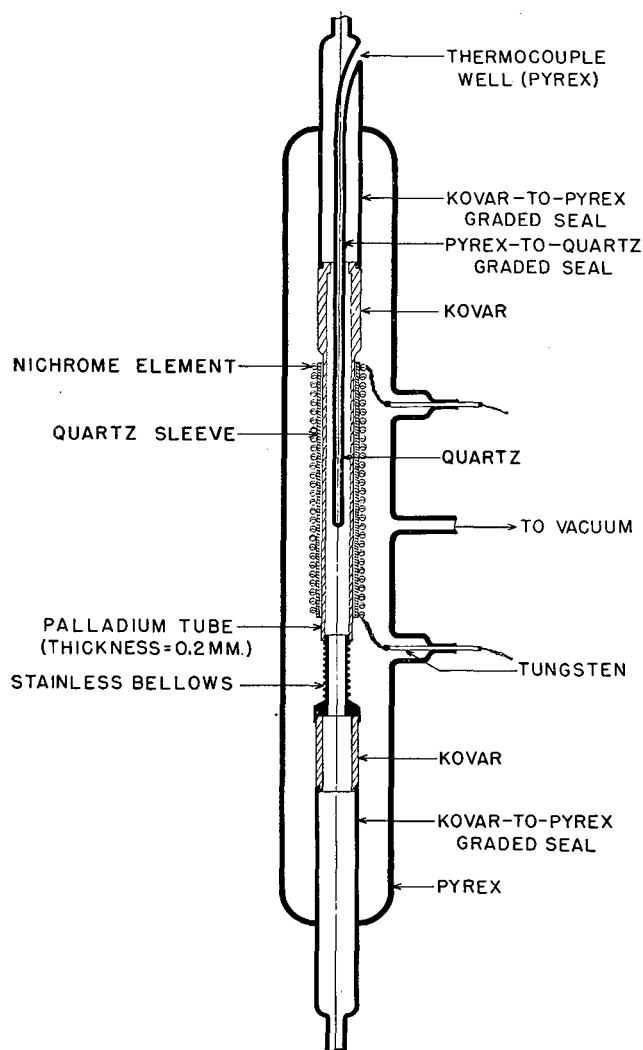
KOVAR

KOVAR-TO-PYREX
GRADED SEAL

PYREX

Figure 3. Hydrogen Diffuser

The thermal conductivity bridge is a laboratory model manu-factured by the Leeds & Northrup Company, Philadelphia, Pa. A milliammeter is placed in series with the two storage batteries supplying current at 12 volts to the bridge and the e.m.f. binding posts are connected to a Brown single-point high speed electronic recorder. The recorder potentiometer has a range of 0 to 80 mv. and indicated voltages can be estimated to ±0.2 mv. The milli-ammeter has a range of 0 to 1000 ma. with an accuracy of 0.5% full scale.

Essentially, the bridge comprises two duplex cells shown as R and X in Figure 1. Cell R, containing helium as the reference gas, may be either evacuated or filled through stopcock $SC6$, one leg of which is connected to the vacuum system. The cell, R, is filled with Matheson cylinder helium (approximately 99.7%) as described below. Both cells are mounted in an oil bath which is maintained at 39° ± 0.1° C.

Outgassing of Carbon. When the apparatus has been as-sembled and is vacuum-tight, the Kanthal furnace is turned on and the quartz converter is opened to the vacuum system through $SC1$. A steady evolution of gas occurs as the furnace is brought up to temperature. Outgassing is continued until a vacuum of 5×10^{-5} mm. of mercury is obtained. Since this requires several hours, it is usually convenient to pump on the carbon overnight. The furnace is then maintained between 1100° and 1150° C.

Thermal Conductivity Calibrations. The thermal conductivity

bridge is zeroed in the following manner. Cylinder helium from Matheson is circulated through the unit and passed into the reference cell, R, until a pressure of 500 mm. of mercury is reached. With the hydrogen diffuser at 450° C. and its jacket under evacuation, the circulatory unit is then filled with helium to about the same pressure, and gas flow is adjusted to a rate of 150 to 200 ml. per minute. The bridge filaments are heated with a current of 550 ma. and zeroed by adjusting the zero rheo-stat until the null point is reached. (This zero setting has re-mained essentially constant over a period of several months de-spite the use of different batches of helium.) The cell, R, is re-filled each time a different cylinder is used.

Determination of points on the calibration curve (Figure 6) for helium–carbon monoxide mixture is carried out by measuring the potential due to mixtures of known composition. These may be made up accurately by volume using the barometric pipet (Figure 4) for introducing a measured amount of carbon mon-oxide or carbon dioxide into the evacuated apparatus. Helium and a small amount of hydrogen are then admitted to a total pressure of 500 to 600 mm. of mercury. The mixture is cir-culated at an arbitrary flow rate of 150 to 200 ml. per minute and when mixing and conversion are complete the potential is read. After measuring the total volume of gas, as described under Volumetric Measurement, the composition of the mixture is calculated. Calibrations may also be made using National Bu-reau of Standards grade benzoic acid. The sensitivity of the bridge at 550 ma. filament current is such that a deflection of about 5 mv. per 1% of carbon monoxide is obtained.

ANALYSIS OF SOLID SAMPLES

In starting a run on a solid sample, the controller is set to maintain 450° C. in the palladium tube, and the line to $SC5$ (Figure 1) is flushed with Matheson tank helium through the mer-cury leg, O. Helium is then admitted to the circulatory unit, grind H is opened, and the carbon bed is continuously flushed with helium while the weighed sample, in a platinum boat, is introduced to the unheated pyrolysis section, I. H is stoppered and $SC5$ is closed. The unit is then evacuated through stopcocks $SC1$ and $SC3$ until the pressure has been reduced to 10^{-3} mm. of mercury or less. $SC1$ and $SC3$ are then turned to permit the circulation of gas, and helium is again admitted until the gage shows a pressure of about 500 mm. of mercury. From the time the sample is introduced until the circulatory unit is refilled with helium, the sample is cooled by an air jet on the pyrolysis tube as a precaution against loss during evacuation.

When helium admission has been completed, the low pressure side of the palladium tube is connected to the vacuum system through $SC7$. Circulation is begun and the gas flow is adjusted to a rate of 150 to 200 ml. per minute. The circuit of the thermal conductivity bridge is closed, the bridge current is adjusted to 550 ma., and the recorder is started.

Decomposition. Power is supplied to the pyrolysis heating element, J, and the current is gradually increased stepwise in such a way as to produce a steady decomposition of the sample. Compounds such as benzoic acid which distill or sublime with-out decomposition probably are not pyrolyzed until they reach the hot carbon bed. On the other hand, polymeric materials and carbohydrates appear to break down partially in the pyrolysis section. The progress of decomposition and conversion is ob-served on the recording potentiometer, and the rate may be ad-justed as required by increasing or decreasing the heat supplied by element J (Figure 1).

Variations in flow rate from 50 to 300 ml. per minute appeared to have no effect on the rate of pyrolysis of benzoic acid. It was therefore concluded that the rate of heating was the controlling factor in the decomposition of most samples. In the authors' ex-perience, however, the accuracy of the results is practically in-dependent of the rate of heating. Consequently, about 80% decomposition may occur within an interval as short as 3 to 5 minutes, as shown in the potential curve of Figure 7. An addi-tional 20 minutes at maximum heat is usually required to com-plete pyrolysis of any residue in the sample boat. Since the circulatory unit has a fixed volume of about 500 ml. at standard temperature and pressure, the rate of decomposition is limited in

the extreme case by the rate at which hydrogen is formed and the rate at which the palladium tube can dispose of it. For samples with molecular weights up to about 500, neither factor assumes any importance. Such samples do not usually exceed 300 mg. in weight, and conversion of the pyrolysis fragments to hydrogen and carbon monoxide is probably accomplished in the first pass over the hot carbon. The efficiency of the palladium tube usually is adequate to prevent the partial pressure of hydrogen from reaching a troublesome magnitude. For example, a 500-mg. sample of polymer with a low oxygen content (5%) is decomposed with little difficulty although over 600 ml. of hydrogen are produced during pyrolysis. However, high polymers of low oxygen content (less than 0.5%) do not yield a high proportion of hydrogen in one pass over the hot carbon at the normal flow rate. Instead, each pass yields, in addition to hydrogen, successively lower molecular weight hydrocarbon fragments with hydrogen as the final product. It is then necessary to increase the contact time—lower flow rate—in order to limit the hydrocarbon concentration in the gas mixture. Under these conditions a higher partial pressure of hydrogen can be maintained, and the diffusion process through the palladium tube can function at a higher efficiency.

Measurement of Bridge Potential. When the bridge potential becomes essentially constant, conversion of all the oxygen in the sample to carbon monoxide is assumed complete. The heater, J, is turned off and the pyrolysis section, I (Figure 1), is allowed to cool for about 10 minutes. At the end of this interval the removal of hydrogen is virtually quantitative, and the residual gas is essentially carbon monoxide in helium. (This was established by mass spectrometer analysis of the residual gas from several runs; in none of these did the amount of hydrogen found exceed 0.2% by volume.) After final adjustment of bridge current to 550 ma. and rate of gas flow to 150 to 200 ml. per minute, the potential on the recorder chart is read. The composition of the helium–carbon monoxide binary is then obtained from a calibration curve. Since the thermal conductivities of hydrogen and helium are of the same order of magnitude, no correction is necessary for the small amount of hydrogen remaining in the mixture.

Volumetric Measurement. Because various parts of the circulatory unit are at different temperatures, the conventional method of measuring the total volume of gas in situ is not practical. The following system of Toepler fractions was adopted for the present research.

As soon as the final potential reading has been obtained, the unit is opened to the barometric pipet, S, through $SC3$, and the mercury level is adjusted to the lowest pointer (Figure 4) after thermal equilibrium has been established. The pressure in the pipet is then recorded, $SC3$ is turned to the vacuum system, and the pipet is re-evacuated. At least two successive fractions of the gas in the unit are removed in this fashion. After the volume in each fraction is calculated the total volume of gas, V_X, is found from the equation, $V_X = (V_A)^2/V_A - V_B$, where V_A and V_B are the volumes of the first and second fractions—all volumes at standard temperature and pressure. Since the derivation of these relations is not readily available in the literature, it is given below.

Consider a system of volume, V, at pressure, p, containing V_X ml. of gas at standard temperature and pressure. Let the gas be distributed among i elements of volume, where each element is at a different temperature so that there are v_1 ml. at standard temperature and pressure in volume V_1 at temperature T_1, v_2 ml. in volume V_2 at T_2, and v_i ml. in V_i at T_i. Then the following relations obtain

$$V_X = \sum^i v_i = \sum^i \frac{pV_i}{T_i} T_0/p_0 \tag{1}$$

where $T_0/p_0 = 273/760$.

The apparent temperature, $\overline{T}$, of the system may then be defined in the equation

$$V_X = \frac{pV}{\overline{T}} T_0/p_0 = \sum^i \frac{pV_i}{T_i} T_0/p_0 \tag{2}$$

If the gas is expanded to a pressure p_a by admitting it to a known volume V_1 which is thermostated at temperature T_1, then

$$V_X = \frac{p_a V}{\overline{T}} \frac{T_0}{p_0} + V_A \tag{3}$$

where

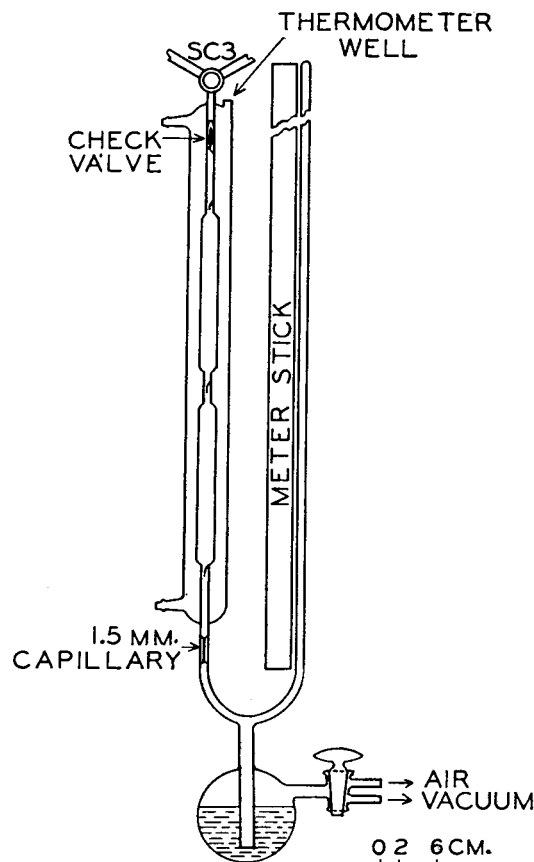$$V_A = \frac{p_a V_1}{T_1} T_0/p_0 \tag{4}$$

Figure 4. Barometric Pipet

If the volume V_1 is evacuated and a second expansion to pressure p_b is made,

$$\frac{p_a V}{\overline{T}} \frac{T_0}{p_0} = \frac{p_b V}{\overline{T}} \frac{T_0}{p_0} + V_B \tag{5}$$

where

$$V_B = \frac{p_b V_1}{T_1} T_0/p_0 \tag{6}$$

On rearrangement, Equation 5 becomes

$$\frac{V}{\overline{T}} = \frac{V_B}{(p_a - p_b)T_0/p_0} \tag{7}$$

and this may be substituted in Equation 3 to give

$$V_X = \frac{p_a V_B}{(p_a - p_b)} + V_A \tag{8}$$

Rewriting Equations 4 and 5 and substituting for p_a and p_b yield an expression which reduces to

$$V_X = \frac{(V_A)^2}{V_A - V_B} \qquad (9)$$

Similarly if a third fraction V_C is removed, it can be shown that

$$V_X = \frac{(V_B)^2}{V_B - V_C} + V_A \qquad (10)$$

These relations are accurate only if the temperature conditions of the apparatus remain substantially constant during any two successive measurements. Agreement within 0.2 to 0.3% relative is usually obtained with volumes calculated from three measurements. Since both the volume of the pipet and the temperature are constant, the calculations are greatly simplified by using an appropriate factor. About 15 minutes are required for three measurements.

An alternative, and possibly easier, procedure will be used in routine determinations to reduce the time required for this measurement to about 5 minutes. In this method, the gas in the circulatory unit will be transferred to a standard thermostated volume and its pressure measured. An automatic Toepler pump should effect this transfer in about fifteen cycles of operation.

Calculation of Results. In Figure 7 is shown the potential curve obtained in a typical determination. The operating conditions, experimental data, and calculations for run 2283-192 on benzoic acid illustrate the method by which the results are computed.

OPERATING DATA.

Initial pressure helium = 499 mm.
Final pressure gas mixture = 567 mm.
Flow rate = 200 ml. per minute
Palladium tube temperature = 450° C.
Reactor temperature = 1130° C.
Bridge current = 550 ma.
Bath temperature (conductivity cell) = 41° C.
Weight of sample = 0.1605 gram
Bridge potential = 62.9 mv. ≡ 11.74% carbon monoxide
Volume, first Toepler fraction = V_A = 109.8 ml. at standard temperature and pressure
Volume, second Toepler fraction = V_B = 85.6 ml. at standard temperature and pressure
Volume, third Toepler fraction = V_C = 66.8 ml. at standard temperature and pressure

CALCULATION. The total volume of gas, V_X, is calculated as follows:

Using Toepler fractions V_A and V_B

$$V_X = \frac{(V_A)^2}{V_A - V_B} = \frac{(109.8)^2}{109.8 - 85.6} = 498.2$$

Using fractions V_A, V_B, and V_C

$$V_X = \frac{(V_B)^2}{V_B - V_C} + V_A = \frac{(85.6)^2}{85.6 - 66.8} + 109.8 = 499.5$$

Mean V_X = 498.9 ± 0.7 ml. at standard temperature and pressure.

From the calibration curve for CXA charcoal the observed potential of 62.9 mv. is equivalent to 11.74% carbon monoxide in the mixture. Then the amount of carbon monoxide due to the oxygen in the sample is 498.9 × 0.1174 = 58.57 ml. at standard temperature and pressure. The percentage of oxygen in the sample is therefore given by

$$\frac{58.57}{22400} \times 16 \times \frac{100}{0.1605} = 26.07\%$$

The theoretical percentage of oxygen in benzoic acid is 26.22%

and the deviation of 26.22 − 26.07 = 0.15% is 0.6% relative to the theoretical percentage.

ANALYSIS OF LIQUID SAMPLES

The weighed evacuated sample tube, 1, of Figure 5, is fi led through the mercury on the sintered disk, 3, with a syringe or pipet which has been drawn out to a fine capillary (11). When it has been reweighed, the tube is attached at grind 4, and grind 5 is joined at F (Figure 1) to the unit through which helium is being flushed. (Once the liquid has been admitted to sample tube 1, the assembly must be kept in a vertical position.) The circulatory unit is then successively evacuated and filled with helium in the manner described for solid samples. During evacuation the sample is cooled in a dry-ice bath; it is afterward warmed to room temperature. The sample is discharged into the circulatory unit by turning the tube and holder about the axis of the pivotal grind 5 (Figure 5) as the mercury runs into 2. Except for liquids of low volatility, little or no warming of the tube is necessary, since the vapor from the sample is carried rapidly into the furnace by the helium stream. In the case of materials of low volatility, the tube is heated in such a way as to maintain a steady distillation of the sample into the carrier stream. Heating portions of the unit between F and I (Figure 1) to prevent condensation of the sample may be done with a torch or with an electric winding.

Analysis of Gas Samples. The procedure followed in the analysis of gases is similar to that described under Thermal Conductivity Calibrations. After measuring the volume of the sample, it is admitted to the evacuated unit through stopcock SC3. Helium is then added and the run is carried out in the usual way. In order to calculate the percentage of oxygen, the density of the sample must be known.

RESULTS

Typical results of oxygen determinations on materials are shown in Tables I, II, and III. Table I gives the data obtained with compounds procured from the National Bureau of Standards. Compounds given in Table II are materials of known purity, chosen to complement the microanalytical standards in Table I. Table III contains the results of determinations on a number of polymeric materials. Polymers differing in functionality are designated by Arabic numerals; different species within a single functional class, by lower-case letters.

These determinations were carried out with J. M. Huber & Company's Aerfloted Arrow carbon black which had an ash content of about 0.02%. By mixing it with 1% graphite this carbon black could be formed into pellets about 4 mm. in diameter and 1 mm. thick. Comparative results (Table IV) on substances from Tables I and II use 4- to 6-mesh pellets of CXA Columbia activated charcoal with an ash content of about 4%.

In general, the accuracy of a single determination on compounds of known oxygen content (Tables I and II) is within the
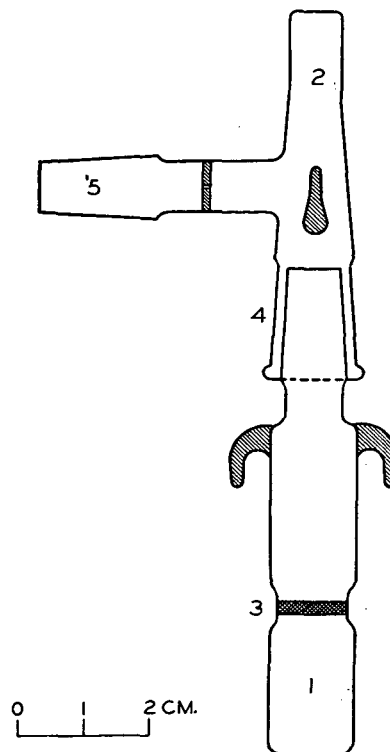

Figure 5. Liquid Sampler

estimated experimental error of 1% relative. The precision of multiple determinations is also about 1% relative on substances of known oxygen content. In the case of polymers, the precision is usually 1%, but may range to 2%, possibly because of inhomogeneities in the samples. The results reported in Tables I, II, and III were chosen at random from the data and are considered typical. For example the seven determinations on benzoic acid shown in Table I were taken from a group of sixteen consecutive runs for which the mean value was 26.26% oxygen with an average deviation of ±0.16% or ±0.6% relative.

The twenty-four substances listed in Tables I, II, and III represent broad differences in oxygen content, molecular structure, functionality, volatility, and molecular weight.

DISCUSSION

The prerequisites to an ideally successful determination of oxygen by the carbon reduction method are quantitative conversion of all the oxygen in the sample to carbon monoxide and exact measurement of the amount of carbon monoxide formed. The problem thus has two aspects—conversion and measurement.

The equilibrium conversion to carbon monoxide at 1100° C. is predicted by thermodynamics to be essentially quantitative (8). However, thermodynamics does not predict the rate at which equilibrium is attained, the extent to which adsorption of carbon monoxide by the charcoal occurs, nor the binding force with which carbon monoxide is adsorbed. It is difficult to assess the influence of these variables on analyses by the flow methods described by previous authors (1–3, 13). Results reported by these authors do not show any noticeable effect of hydrogen to oxygen ratio on conversions, possibly because the relative partial pressure of hydrogen is low during the time pyrolysis fragments containing oxygen are in contact with the carbon. The kinetic factor was recognized by Aluise and his co-workers (1) who obtained poor conversions with both graphite and a lamp black charcoal. Attributing the poor conversions to low surface areas, these authors recommended an amorphous type carbon which was ash free. This was the first departure from the concept (4) that the carbon is merely a contact mass.
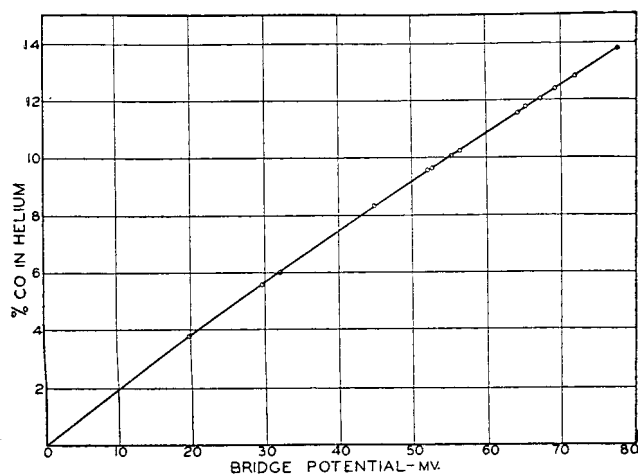

Figure 6. Calibration Curve for He-CO Mixtures (Huber's Carbon Black)

It is clear from the work of Hinshelwood's collaborators (6) that the portion of the carbon surface entering into the water gas and carbon dioxide conversions in the region of 750° C. is highly specific. It seems reasonable to assume that the same situation holds true at 1150° C., although a larger fraction of the surface is undoubtedly involved. These authors (6) found that both processes followed the usual type expression for a heterogeneous reaction retarded by its products. For example, the rate of the steam-carbon reaction is given by the relation

$$\text{rate} = \frac{k_1 p_{H_2O}}{1 + k_2 p_{H_2} + k_3 p_{H_2O}} \tag{11}$$

This reaction is therefore retarded by adsorbed hydrogen. In a similar fashion the rate of the carbon dioxide–carbon reaction is
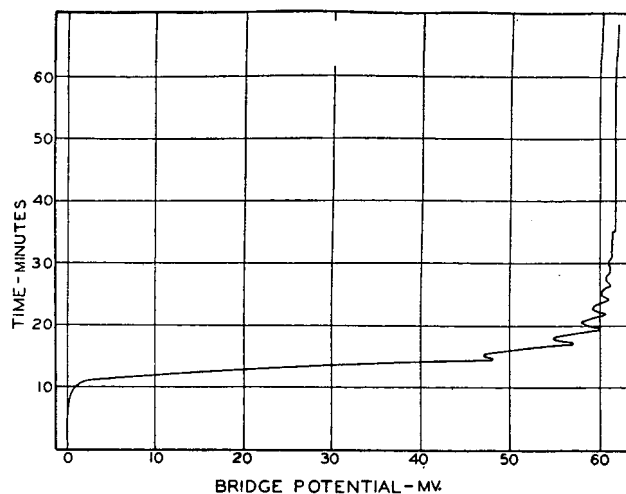

Figure 7. Potential Curve for Typical Determination

reduced by both hydrogen and carbon monoxide. Although the authors have not specifically investigated the effect of hydrogen on reaction rate under the conditions of this investigation, it has been observed qualitatively that the rate of conversion is reduced appreciably after pure hydrogen has been circulated over the carbon. Using standard helium–carbon dioxide mixtures from a cylinder the normal rate of conversion was rapid but not instantaneous.

All these considerations entered into the decision to use a closed circulating system and to remove hydrogen by selective diffusion through a heated palladium membrane. Circulation of the pyrolysis products over the carbon ensures the formation of the equilibrium concentration of carbon monoxide, while the removal of hydrogen shifts the equilibrium toward a more favorable carbon monoxide conversion. At the same time hydrogen removal appears advantageous from a kinetic standpoint.

Since the removal of hydrogen from the circulatory unit creates a binary gas mixture, any one of several instrumental methods could be used to determine the composition of the helium–carbon monoxide mixture with considerable accuracy. Although the thermal conductivity method was chosen because of its relative simplicity, infrared and possible interferometric analysis should also be satisfactory. (If infrared analysis were chosen, the method should be applicable without further modification to compounds containing nitrogen and halogens.) In consequence, the inherent difficulties in the determination of carbon monoxide by chemical reaction are avoided.

Additional advantages also derive from the use of instrumental methods. Since the instrument is calibrated in position, with gas mixtures of known composition, the effects of impurities in the carrier gas are eliminated and intensive purification of the carrier is no longer necessary. Secondly, if it is assumed that the carbon surface is consistently reproducible, the effect of carbon monoxide adsorption is also eliminated by calibration. On the basis of periodic check runs using the standard mixtures of carbon dioxide in helium, the assumption of a reproducible carbon surface is justified where the Huber carbon is concerned. Although the data in Table IV on CXA Columbia activated carbon indicate that good results can be obtained with a high ash charcoal, this carbon was stable for only a limited time. The carbon could be evacuated only with difficulty and during its use some components of the ash distilled to the end of the quartz reactor. Coupled with the erratic results obtained both before and after the stable period, these observations suggest the presence of a variable surface complex which probably involves the carbon oxides and the ash components. Thus, the erratic behavior of the CXA charcoal might be attributed to sorption-desorption processes associated with this surface complex.

Probably the chief advantage of an instrumental method, such

Table I. Microanalytical Standards from National Bureau of Standards

Compd.	Run No.	Sample Wt., Mg.	CO in He Mixt., %	Oxygen, % Calcd.	Found	Abs. error
Benzoic acid	60-54	139.8	10.38	26.22	25.94	-0.28
	60-56	173.6	12.88	...	26.26	+0.04
	60-58	155.2	11.56	...	26.25	+0.03
	60-60	174.6	12.08	...	26.22	0.00
	60-62	70.5	5.60	...	26.39	+0.17
	60-64	191.1	13.84	...	26.02	-0.20
	60-68	50.5	3.80	...	26.15	-0.07
Anisic acid	60-122	111.9	10.08	31.55	31.68	+0.13
	60-124	122.2	10.84	...	31.78	+0.23
	60-126	58.5	5.56	...	32.06	+0.51
Dextrose	60-170	74.3	11.08	53.29	53.84	+0.55
	60-174	75.0	11.26	...	53.02	-0.27
Sucrose	60-166	75.1	11.76	51.43	52.07	+0.64
	60-168	80.8	11.68	...	51.49	+0.06
	60-172	76.0	11.12	...	51.21	-0.22

Table II. Laboratory Standards

Compd.	Run No.	Sample Wt., Mg.	CO in He Mixt., %	Oxygen, % Calcd.	Found	Abs. error
Adipic acid	60-130	111.8	13.40	43.81	43.71	-0.10
	05-34	105.0	12.80	...	43.48	-0.33
	05-36	97.8	11.72	...	44.08	+0.27
	05-40	72.7	9.52	...	43.88	+0.07
Benzil	60-182	266.1	11.58	15.22	15.42	+0.20
	05-58	242.1	10.84	...	15.32	+0.10
	05-60	55.9	1.98	...	14.94	-0.28
Coumarin	05-98	171.3	10.56	21.91	22.10	+0.19
	05-100	160.6	10.21	...	21.91	0.00
	05-102	182.9	11.42	...	22.01	+0.10
Dimethyl ether	60-194	(43.71)[a]	10.24	34.73	34.90	+0.17
	05-136	(48.81)[a]	11.60	...	34.72	-0.01
	05-138	(39.73)[a]	9.68	...	34.53	-0.20
n-Decyl adipate	05-140	204.4	8.90	15.00	14.87	-0.13
	05-146	212.7	9.84	...	15.40	+0.40
Ethyl alcohol[b]	05-16	99.5	10.40	34.73	34.89	+0.16
	05-18	117.5	11.62	...	35.23	+0.50
	05-20	74.7	7.67	...	35.18	+0.45
Glutaric acid	05-78	113.1	14.80	48.45	48.31	-0.14
	05-70	117.5	15.38	...	48.42	-0.03
	05-68	95.4	12.98	...	48.84	+0.39
	05-66	90.0	12.10	...	48.31	-0.14
Vanillin	60-176	145.7	11.68	31.55	31.16	-0.39
	60-178	105.9	9.80	...	31.82	+0.27
	05-80	157.4	13.46	...	31.64	+0.09

[a] Milliliters at standard temperature and pressure.
[b] Contained 0.25% water.

as thermal conductivity, is that changes in composition may be continuously recorded. The value of being able to follow events in the reaction system may be illustrated by experience with polythene. In the early runs on 3-gram samples of this polymer, the bridge potential was clearly dependent on the rate of decomposition of the sample during pyrolysis. As the rate increased, the bridge potential increased. If the heat supplied to the pyrolysis section was reduced, the bridge potential decreased. This indicated the transient existence of volatile hydrocarbons in the gas from the hot carbon reactor. Evidently ultimate decomposition of polythene fragments was not occurring in a single pass over the carbon and a reduction in flow rate was necessary during decomposition. The rate of hydrogen diffusion is dependent on the partial pressure of hydrogen according to the relation (9),

$$D = \frac{K}{d}\, p^{1/2}\, e^{-E_0/2kT} \tag{12}$$

Then since the total pressure which the system may attain is limited, the rate at which hydrogen diffuses through the palladium membrane is seriously reduced when the relative concentration, in the gas mixture, of hydrocarbons to hydrogen is large. The general advantages of a knowledge of the progress of decomposition of an unknown sample are evident. These results show that

the method of decomposition of a sample may have a real effect on the ease and rapidity with which it can be run.

The closed circulatory unit permits the use of vacuum techniques in the introduction of liquid and gas samples. At the same time, evacuation of the carbon to pressures of less than 1 micron stabilizes its surface. The existence of adsorbed gases in the form of oxide complexes at temperatures as high as 1200° C. has been described by Emmett (5). His work shows that there are a number of complexes with varying degrees of stability, some of which can be removed only by evacuation in the 900° to 1200° C. range. For this reason preliminary evacuation of the system before each run was considered desirable.

The time required for a single oxygen determination is usually about an hour, but when samples weighing over 500 mg. are to be run, a longer time is necessary. The ability of the palladium tube to diffuse hydrogen rapidly makes it practicable to run samples as large as 3 grams. By using a sample of this size and a recorder with a range of 0 to 10 mv. it should be practicable to determine oxygen contents as small as 0.01% within 5% relative or better.

At present the method is limited to the determination of oxygen in substances containing only carbon, hydrogen, and oxygen. However, preliminary results suggest that the general technique can be extended to the simultaneous determination of hydrogen and nitrogen as well as oxygen, or by substituting copper oxide

Table III. Polymeric Materials

Designation	Functional Type	Run No.	Sample Wt., Mg.	CO in He, %	Oxygen, % Found	Mean	Av. dev.
Polymer 1a	Ketone	60-150	656.3	8.30	4.37		
		60-152	493.5	6.28	4.38	4.38	0.01
Polymer 1b	Ketone	60-132	512.6	6.44	4.53		
		60-134	556.6	7.16	4.25	4.39	0.14
Polymer 2a	Air-blown unsaturate	60-154	218.3	4.60	7.05		
		60-156	281.7	5.92	7.02	7.04	0.02
Polymer 2b	Air-blown unsaturate	05-114	206.4	14.90	25.09		
		05-116	198.4	14.38	25.31	25.20	0.11
Polymer 3a	Ester	60-142	494.0	9.92	6.47		
		60-144	494.4	9.76	6.56	6.51	0.05
Polymer 3b	Ester	60-136	441.6	7.80	6.18		
		60-138	478.9	9.30	5.95		
		60-140	475.4	9.20	6.13	6.09	0.09
Polymer 4a	Carbinol	05-106	211.9	11.58	17.10		
		05-108	205.0	11.24	16.98	17.04	0.06
Polymer 4b	Carbinol	05-110	244.3	13.04	16.86		
		05-112	224.6	11.88	17.14	17.00	0.14
Polymer 5a	Formal	05-128	116.9	14.28	43.57		
		05-130	117.0	14.05	43.53	43.55	0.02
Polymer 6a	Hydrocarbon	05-120	1282.4	3.34	0.76		
		05-122	1305.2	3.32	0.73	0.75	0.02
Polymer 6b	Hydrocarbon	05-124	1289.1	5.64	1.38		
		05-126	1301.1	5.56	1.38	1.38	0.00
Polymer 7a	Hydrocarbon	60-158	529.5	0.20	0.121		
		60-160	525.6	0.26	0.120	0.12	0.00

Table IV. Samples Run on CXA Charcoal

Compd.	Run No.	Sample Wt., Mg.	CO in He Mixt., %	Oxygen, % Calcd.	Found	Abs. error
Adipic acid	2283-146	121.4	14.18	43.81	43.28	-0.53
	2283-188	109.4	12.93	...	43.72	-0.09
	2283-190	102.5	12.56	...	44.47	+0.66
Benzoic acid	2283-138	155.3	11.26	26.22	26.15	-0.07
	2283-164	103.4	7.85	...	26.44	+0.22
	2283-192	160.5	11.74	...	26.07	-0.15
Benzil	2283-198	304.3	12.78	15.22	15.24	+0.02
	2360-2	309.5	12.52	...	15.17	-0.05
	2360-4	307.3	12.98	...	15.36	+0.14
Glutaric acid	2283-112	86.4	11.81	48.45	48.26	-0.19
	2283-172	107.5	13.66	...	48.60	+0.15
	2283-174	107.7	14.41	...	48.55	+0.10
Dextrose	2283-86	28.5	4.66	53.29	54.09	+0.80
	2283-92	43.3	6.88	...	53.50	+0.21
	2283-180	79.9	11.52	...	52.54	-0.75
Vanillin	2283-98	97.8	8.88	31.55	31.08	-0.47
	2283-100	153.1	13.32	...	31.14	-0.41
	2283-134	123.5	11.22	...	32.24	+0.69

for carbon in the converter, to the simultaneous determination of carbon, hydrogen, and nitrogen. It is hoped that interference from halogens or sulfur can be eliminated without undue difficulty.

LITERATURE CITED

(1) Aluise, V. A., Hall, R. T., Staats, F. C., and Becker, W. W., ANAL. CHEM., 19, 347 (1947).
(2) Chambers, W. T., paper presented at the Rubber Technology Conference, London (June 23–25, 1948).
(3) Dinerstein, R. A., and Klipp, R. W., ANAL. CHEM., 21, 545 (1949).
(4) Elving, P. J., and Ligett, W. B., Chem. Revs., 34, 129 (1944).
(5) Emmett, P. H., Ibid., 43, 69 (1948).
(6) Gadsby, J., Long, F. J., et al., Proc. Roy. Soc. (London), A187, 129 (1946); A193, 357, 377 (1948).
(7) Kirner, W. R., IND. ENG. CHEM., ANAL. ED., 6, 358 (1934).
(8) Wagman, D. D., Kilpatrick, J. E., Taylor, W. J., Pitzer, K. S., and Rossini, F. D., J. Research Natl. Bur. Standards, 34, 143 (1945).
(9) Smithells, C. J., "Gases and Metals," p. 94, New York, John Wiley & Sons, 1937.
(10) Meulen, H. ter, Chem. Weekblad, 19, 191 (1922).
(11) Taylor, R. C., and Young, W. S., IND. ENG. CHEM., ANAL. ED., 17, 811 (1945).
(12) Unterzaucher, J., Ber., 73B, 391 (1940).
(13) Walton, W. W., McCulloch, F. W., and Smith, W. H., J. Research Natl. Bur. Standards, 40, 443 (1948).

Calorimeter for Some Corrosive Liquids

B. H. SAGE AND E. W. HOUGH[1]

California Institute of Technology, Pasadena 4, Calif.

The calorimeter described is suitable for measuring the heat capacity of corrosive liquids at temperatures up to 400° F. This instrument follows the general design utilized by Osborne and co-workers for the study of the heat capacity of water. The calorimeter appears to be capable of yielding results with an uncertainty of less than 1%, provided the rates of corrosion and decomposition of the fluid under investigation are sufficiently small. The calorimeter was constructed of stainless steel containing a large amount of chromium and nickel and was gold-plated on the internal surfaces to decrease further the rate of corrosion by materials such as red fuming nitric acid. Agitation was provided in the calorimeter to ensure thermal and phase equilibrium, and the assembly was confined within an adiabatic vacuum jacket. Provisions were made for the addition electrically of known amounts of energy to the calorimeter and its contents and for measurements of the resulting change in state. From these and other data, the heat capacity of the liquid was established.

THE determination of the heat capacity of fluids by calorimetric techniques is not new. The general principles of such measurements were presented by White (13), who discussed the more essential features of calorimeters and indicated the sources of error. It is believed that the greatest advances in the adaptation of these techniques to use at elevated pressures were made by Osborne and co-workers (5, 6), who developed a modern calorimeter capable of establishing the heat capacity and enthalpy of the saturated liquid and the enthalpy change upon vaporization of a pure substance over a wide range of temperatures.

The calorimeter and associated equipment, the general features of which were similar to those employed by Osborne (5, 6), are shown schematically in Figure 1.

The assembly consisted of a vacuum jacket, A, within which the calorimeter, B, was suspended by small wires. A diffusion-type vacuum pump, C, with an appropriate forepump was connected to the vacuum jacket at D. A small centrifugal-type impeller was mounted at E within the calorimeter and served to agitate the contents. A connection from the bottom of the calorimeter to appropriate equipment, F, was used to measure the pressure. The temperature of the bomb and its contents was determined by means of a resistance thermometer, G (Figure 1).

The operation of the equipment involved the addition of a known weight of the material under investigation and the establishment of the energy added electrically by the heater, H, to raise the temperature of the calorimeter and contents a known amount. The rise in temperature was determined by means of resistance thermometer G. Significant gains or losses of energy from the calorimeter were prevented by maintaining the wall of jacket A at substantially the same temperature as the exterior

of calorimeter B. Any difference in the temperatures of the two surfaces was ascertained by means of copper-constantan thermocouples.

From a sequence of measured increments in temperature, each of which resulted from the addition of a known amount of energy, the change in internal energy of the calorimeter and contents was established as a function of temperature. Upon the completion of such a set of measurements, the quantity of material in the calorimeter could be altered and the measurements repeated. From two such series, the change in the internal energy of the fluid in the calorimeter could be determined without knowledge of the heat capacity of the calorimeter or of the nuisance volumes (1). The measurements were carried out in the heterogeneous region and corrections were made for the changes in phase associated with the change in temperature of the calorimeter and contents. However, in order to avoid the need for two sets of measurements with unusually corrosive materials, procedures requiring knowledge of the heat capacity of the colorimeter and nuisance volume were often employed.

METHODS OF MEASUREMENT

The evaluation of the heat capacity of a fluid by the methods just discussed consisted of two principal steps: first, evaluation of the net quantity of energy added to the calorimeter and contents and, second, determination of the resulting change in state. The first step involved little that was thermodynamic in nature, inasmuch as it included the determination of energy transfer to and from the calorimeter and the means of evaluating the energy added electrically to the equipment. The second step was primarily a thermodynamic analysis of the resulting processes.

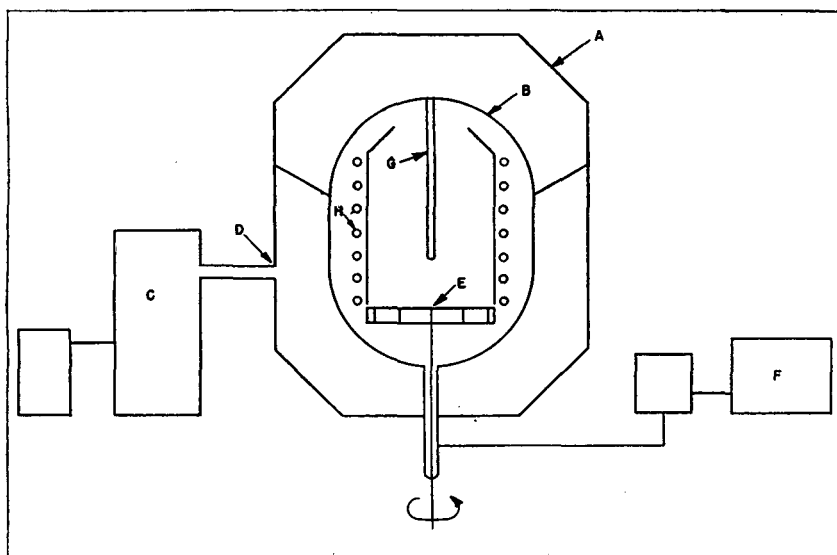[1] Present address, Stanolind Oil and Gas Company, Tulsa, Okla.

Figure 1. Schematic Arrangement of Calorimeter

of the calorimeter was established from two sets of measurements upon water, utilizing the enthalpy data of Osborne and co-workers (2, 5, 7). Good agreement was obtained between the present set of heat capacity measurements and those of Osborne et al. (7).

DESCRIPTION OF APPARATUS

The general arrangement of the calorimeter assembly is shown in Figure 2.

The calorimeter, A, was constructed of stainless steel of moderately high tensile strength and was machined in two parts to be joined by a tapered acme thread. Figure 3 is a photograph of the calorimeter prior to assembly. The vessel was designed for an operating pressure of 1000 pounds per square inch, at which pressure the steel was stressed to a maximum value of 20,000 pounds per square inch, well below the ultimate strength of the metal. The interior of the calorimeter was plated with gold to decrease the rate of corrosion by the material under investigation. The total thickness of gold of approximately 0.015 inch was applied in five successive operations. The gold was burnished between each plating to attempt to fill the small pores. It is probable that even with this laminated structure of the gold plate a substantial part of the corrosion of the calorimeter with nitric acid resulted from its reaction with the stainless steel through pores. Lugs were provided on the outside of the bomb to facilitate assembly and disassembly and to reinforce the spherical walls at the points where the leads to the internal heater were brought through the wall of the calorimeter. Agitation within the calorimeter was obtained by means of the impeller, B, Figure 2, which induced circulation through the ports, C, over a heater which is not shown, and up around the circulation shield, D. The fluid flowed down around the resistance thermometer, E, and returned to the impeller along the radial guide vanes, F. Provision also was made to circulate a small part of the total flow through the space below impeller B.

A photograph of the principal internal parts of the calorimeter including the impeller, shield, and guide vanes is shown in Figure 4. These parts were also coated with a fairly heavy gold plate.
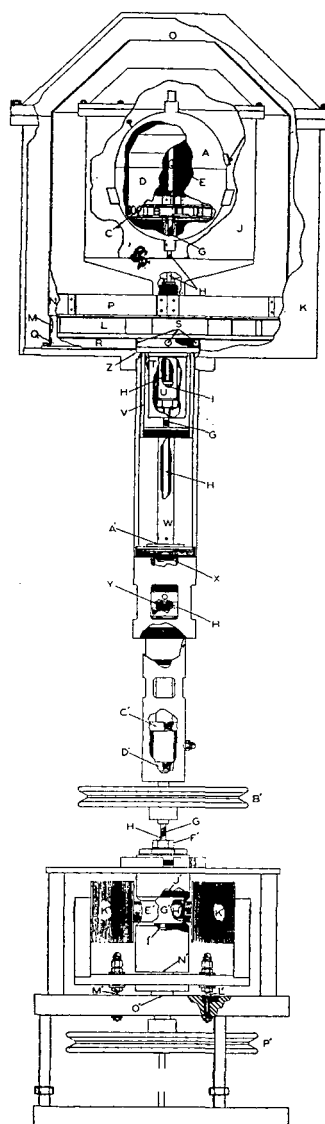
The methods employed in establishing the net quantities of energy added to the calorimeter were similar to those used by Osborne and co-workers (5, 6) and followed by other investigators in this field (9) with but slight revision. Corrections were made in the conventional fashion for the energy loss from the calorimeter as the result of radiation and for the gain in energy associated with the mechanical agitation of the contents. These determinations in conjunction with the measured electrical input yielded values of the net energy additions with an uncertainty of not more than 0.1%.

The quantity of material in the calorimeter was considered to be constant for a particular loading, as the change in weight of material within the connecting lines was negligible. This variation in weight might have been taken into account, but it materially complicated the calculation of the results without significantly increasing their accuracy. This "nuisance volume" was less than 2% of the total volume of the calorimeter and was filled with liquid. In these circumstances it appeared that a change in weight of material within the calorimeter during a set of measurements was usually less than 0.05% of the total material present, thus permitting an average value to be used safely.

One of the more accurate means of evaluating thermodynamic properties involved similar measurements with two different quantities of material in the system. This approach was used by Osborne (6) and others (12) to good advantage. The basic expression was presented recently (12) for evaluating the change in internal energy of the calorimeter and contents in terms of the properties of the phases and the relationships applicable to the estimation of the isobaric heat capacity. This method, when applied to a pure substance, necessitated measurements with two rather widely different quantities of the liquid and gas phases present, as well as data concerning the volumetric properties of each phase. Such information need not be highly accurate and in many instances may be estimated satisfactorily from the law of corresponding states. The calorimeter described here was not well suited to the measurement of the heat capacity of gases, except those of fairly high molecular weight at elevated pressures.

The technique outlined above cannot be used in some instances without excessive deterioration of the calorimeter because of the lengthy contact of the corrosive fluids with the apparatus. However, the heat capacity of the calorimeter may be ascertained, thus eliminating the need for more than a single set of measurements. This procedure is believed to be somewhat less desirable, but was followed by Osborne and Van Dusen (8) and others (10). In the present instance the apparent heat capacity

Figure 2. General Arrangement of Calorimeter
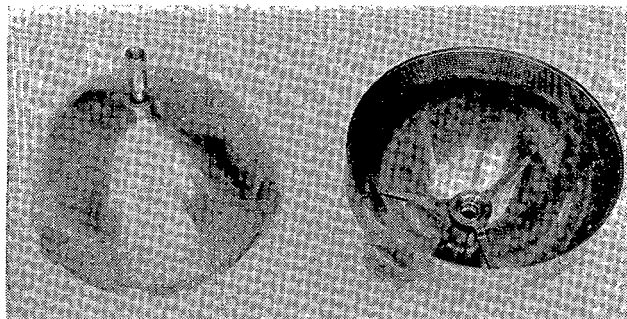
The rate of corrosion of the AISA Type 320

Figure 3. Calorimeter Bomb Prior to Assembly

stainless steel employed in the construction of the calorimeter under the action of red fuming nitric acid was established as a function of temperature. This rate was determined by a series of measurements at a given temperature, from which the rate of loss of the stainless steel with time was fixed. The influence of separation and drying followed by re-exposure was not large. Most of the exposure times were for a period of 24 hours. It was found that the average corrosion rate at 235° F. was approximately 0.71 inch per year at a volume-to-area ratio of 1 inch. At this rate, it would be difficult to obtain accurate heat capacity data because of the enthalpy changes resulting from the reaction between red fuming nitric acid and stainless steel. It is also necessary to avoid rapid deterioration of the calorimeter. The corrosion rate of red fuming nitric acid upon a gold-plated sample of this stainless steel at 235° F. was 0.21 inch per year at about the same volume-to-area ratio. This rate represents the conditions after approximately 36 hours' exposure.

The impeller, B, Figure 2, was driven by the shaft, G, enclosed in tube H which emerged from the vacuum jacket through a seal, I. B was mounted upon platinum-glass bearings shown in Figure 2. Small ports that do not appear in this figure were used to connect the annular space between shaft G and the interior of tube H with the lower part of the calorimeter. These ports were so located that substantially all the liquid in the calorimeter could be removed through H.

A string-free platinum resistance thermometer within the stainless steel tube, E, Figure 2, was used to measure the temperature of the calorimeter and contents. Conventional four-lead connections from this resistance thermometer to a Mueller bridge were brought out of the vacuum jacket, J, through individual tubes for each lead. The entire calorimeter was assembled with block tin because of the fairly low corrosion rate of this material in contact with red fuming nitric acid. The calorimeter was supported within J by means of three small wires.

The jacket, J, Figure 2, was constructed of stainless steel and immersed in the oil bath, K. The impeller, L, discharged the oil through the ports, M, and circulated it around the outside of the shield, N, upon which two electric heaters were mounted to aid in the control of the temperature of the oil bath. The flow of the oil was upward around N, through the opening, O, down around the vacuum jacket, past the guide vanes, P, and to the eye of the impeller, L. Some auxiliary circulation was provided to bring the temperature of H to that of J. This accessory flow was directed inward through ports Q, along the guide vanes, R, through ports S, and downward past the shield, T. The flow then returned inside T, upward past the sleeve, U, and thence to the inlet of L. Drive for the oil bath impeller, L, was obtained through the sleeves, V and W. Sleeve W emerged from the oil bath through the packing shown at X, Figure 2. Tube H, which was stationary, passed through the packing, Y. Impeller L and sleeves V and W were supported upon bearings at Z and A'. Z provided support for both axial and radial thrust. The pulley, B', which was supported by bearings C' and D', was used to drive L. H was sealed to the shell, E', just below the hexagonal nut, F'. Shaft G was connected to the stainless-steel armature housing, G', which rotated on small platinum-glass bearings located at I' and J' in the stainless-steel shell, E'. The armature, H', was driven by means of two electromagnets, K', which rotated around the axis of the equipment. These electromagnets were energized through slip ring contacts L' and M'. The electromagnet assembly was supported by bearings at N' and O' and driven by pulley P'. The oil bath with the impeller installed is shown in Figure 5.

The oil bath, K, Figure 2, was surrounded by an adiabatic jacket, which is not shown and which was maintained by manual control at substantially the same temperature as the oil bath. This shield was desirable in order to avoid the need for the large energy additions which otherwise would be required to maintain the oil bath at the appropriate temperature. Photographs of the magnetic drive for the calorimeter agitator and the belt drive for the oil bath agitator appear in Figure 6. The magnetic drive was operated at approximately 200 r.p.m. and afforded adequate torque to rotate the impeller at this speed.

The entire calorimeter was housed in a separately ventilated structure in order to prevent damage to other parts of the laboratory and to personnel in case of a complete failure of the apparatus when being operated at elevated temperatures. Figure 7 indicates the general arrangement of the calorimeter with the associated control equipment. The calorimeter was placed upon a steel frame covered inside and out with Transite sheeting, and the control room, which was adjacent to the calorimeter, housed almost all of the electrical control equipment required. The calorimeter was located in the room which comprised the right half of the housing shown in Figure 7. This room was maintained at a slightly reduced pressure by a blower discharging to the atmosphere.

Various other pieces of apparatus were contained in the left-hand room shown in Figure 7. The mechanical vacuum pump and a three-stage jet pump, with capacities of about 0.15 and 8 cubic feet per second, respectively, were used to evacuate the adiabatic jacket of the calorimeter. One of the potentiometers was used in conjunction with a thermocouple, a galvanometer, a photoelectric circuit, and a light source to govern the temperature of the oil bath. The difference in temperature between the oil bath, A, and the calorimeter, B, Figure 1, was kept small enough by this arrangement to minimize thermal transfer. The energy for the internal calorimeter heater was furnished electrically by four 6-volt storage batteries. The potential difference across and the current through the heater were measured by a Type K-2 potentiometer shown in Figure 7. The temperature difference between the adiabatic jacket and the oil bath was measured by a White double potentiometer. The resistances of the two platinum resistance thermometers in the calorimeter were determined by a Mueller-type bridge.

The material was added to the calorimeter through a tube entering the bottom of E', Figure 2. Conventional high-vacuum techniques utilizing weighing bombs (9) were employed in adding or withdrawing material. It is believed that these techniques have been so refined that weight of material added to the calorimeter was determined with an uncertainty of less than 0.8%.
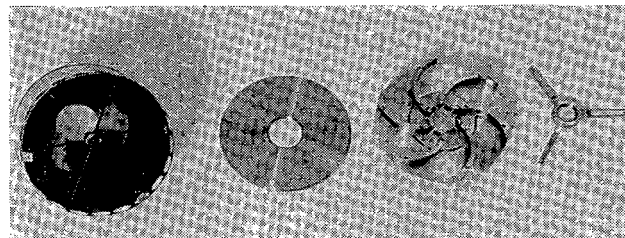

Figure 4. Internal Parts of Calorimeter Bomb

The pressure within the calorimeter was established through a stainless-steel diaphragm attached to E' and oil-filled tubing which led to a pressure balance (11). The stainless-steel diaphragm was necessary in order to avoid reaction between the materials under investigation and the oil in the pressure balance. It appeared that, by appropriate control of the position of the diaphragm by means of an electric contact, no significant additional uncertainty in pressure measurement was introduced by its use. A description of the principles of operation and the methods of calibration of this equipment is available (11).

CALIBRATION

The details of calibration, particularly regarding such quantities as pressure, temperature, rate of thermal transfer between calorimeter and jacket, and energy added through agitation of the contents of the calorimeter, are important in the use of the instrument and require special discussion. In the case of corrosive liquids, where it is not practical to carry out two sets of measurements involving different weights of sample, a knowledge of the nuisance volume and of the heat capacity of the calorimeter is necessary.

The pressure balance was calibrated against the vapor pressure of carefully purified carbon dioxide at the ice point (*1*). Calibrations of this instrument for nearly a decade indicated that the pressures were known in relation to this standard with an uncertainty of not more than 0.1% or 0.2 pound per square inch, whichever was larger.

The temperature of the calorimeter was measured with a strain-free platinum resistance thermometer and uncertainties greater than 0.05° F. relative to the International Platinum Scale were unlikely. The platinum resistance thermometer was compared with the indications of a similar instrument that had been calibrated by the National Bureau of Standards. The change in temperature for a single energy addition, which was about 6° F., was measured with an uncertainty of not more than 0.004° F. Corrections were made for variations in the temperature of the Mueller bridge used in measuring the resistance of the thermometer.

The procedure followed in connection with the determination of the rate of energy transfer between the calorimeter bomb and the jacket was described above. At temperatures near 100° F. the uncertainty in evaluating the thermal transfer between the calorimeter and jacket was reduced to less than 0.22% of the total energy added. At the higher temperatures this uncertainty was probably about 0.3% because of the increased importance of radiation in the thermal transfer under such conditions.

In the case of liquids of relatively low viscosity, the total energy added by means of the calorimeter agitator during the heating and equilibrium periods was less than 1% of the energy added electrically. It has been found from other calorimeters of similar design that the energy added as a result of the agitation may be ascertained with an over-all uncertainty of approximately 3%, corresponding to an uncertainty in the heat capacity measurement of not more than 0.05%. However, it was necessary to carry out a calibration of the extent of the energy additions resulting from agitation for each system at four or more temperatures within the range between 100° and 460° F. These calibrations were necessary as a result of the change in viscosity and specific weight of the liquid phase with temperature. The rate of

temperature rise with the agitator in operation was measured under substantially adiabatic conditions with no energy added electrically to the calorimeter. The impeller was driven, at the same speed, during both calibration and use because the rate of energy addition was markedly influenced by this variable.

For determinations involving only a single weight of sample, additional information concerning the character of the calorimeter was required. Its volume was determined by adding known weights of methane and by measuring the resulting equilibrium pressure at a known temperature. This procedure established the total volume of the bomb, including the nuisance volume. The latter was independently determined by adding a hydrocarbon oil of known specific weight to the evacuated nuisance space and ascertaining the weight of liquid required to fill it. The volume of the calorimeter was 0.043510 cubic foot at 100° F., and the nuisance volume was 0.000811 cubic foot.

The heat capacity of the calorimeter was determined (*13*) by measurements with two samples of water. Accurate thermodynamic data were available (*2, 5, 7*) for this purpose.

From measurable quantities and the known thermodynamic properties of water, the following equation serves to evaluate the total internal energy of the calorimeter as a function of temperature:

$$Q = \int_{\theta_1}^{\theta_2} \left\{ m_d \left[C_{Pd} \frac{dT}{d\theta} d\theta - P'' \left(\frac{\partial V_d}{\partial T} \right)_P \frac{dT}{d\theta} d\theta - T \left(\frac{\partial V_d}{\partial T} \right)_P \frac{dP}{d\theta} d\theta - P'' \left(\frac{\partial V_d}{\partial P} \right)_T \frac{dP}{d\theta} d\theta \right] + \right.$$
$$(m - m_d) \left[C_{Pb} \frac{dT}{d\theta} d\theta - P'' \left(\frac{\partial V_b}{\partial T} \right)_P \frac{dT}{d\theta} d\theta - T \left(\frac{\partial V_b}{\partial T} \right)_P \frac{dP}{d\theta} d\theta - P'' \left(\frac{\partial V_b}{\partial P} \right)_T \frac{dP}{d\theta} d\theta \right] +$$
$$\left. \left[T(V_d - V_b) \frac{dP''}{dT} + P''(V_b - V_d) \right] \frac{dm_d}{d\theta} d\theta + \frac{dE_B}{dT} \frac{dT}{d\theta} d\theta \right\} \quad (1)$$

Good agreement with the heat capacity data of Osborne (*7*) was obtained from the two sets of measurements with water. From a review of the accuracy of the internal energy data for water (*6, 7*), it appeared that the heat capacity of the calorimeter could be established with a probable error of not more than 0.25% at temperatures below 300° F. Above this temperature the uncertainties would increase and reach a value of about 0.5% at 460° F. In the case of materials having heat capacities comparable to those of water, the uncertainty of 0.25% in the heat capacity of the calorimeter will cause only about 0.2% uncertainty in the heat capacity of the material.

The over-all accuracy of electrical measurements was relatively high, and the uncertainty of the integrated values of the energy added to the calorimeter was probably not more than 0.04%. The primary uncertainty in this quantity rested with the measurement of time because the frequency of the alternating current in the laboratory was taken as a standard. Probable uncertainties of about 0.1 second were to be expected in any elapsed time measured by this method. If further refinements in the heat-capacity measurements were needed, it is believed that the next step would involve the establishment of a more precise timing system.

The estimated uncertainty in the calibration of the several quantities of direct interest in determining the over-all accuracy of the heat-capacity measurements is listed in the following table:

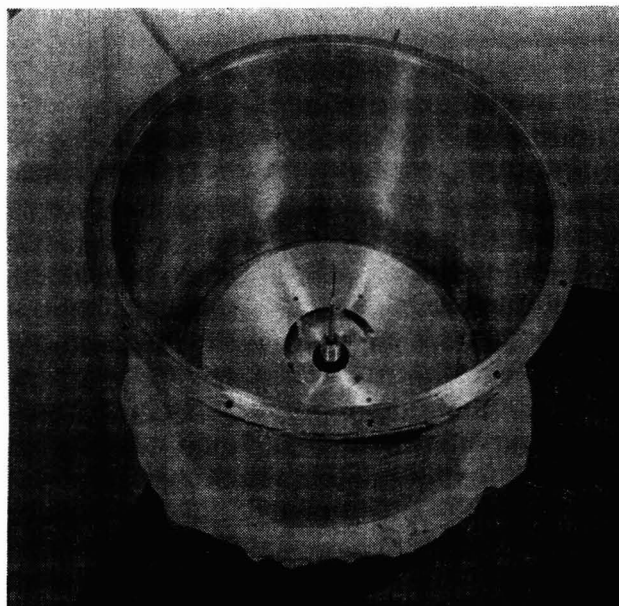| Quantity	Probable Uncertainty, %	
	Method A	Method B
Change in temperature	0.08	0.08
Energy exchange between calorimeter and jacket	0.22	0.22
Agitation	0.05	0.05
Nuisance weight	...	0.10
Heat capacity of calorimeter	...	0.25
Energy added electrically	0.04	0.04

Figure 5. Oil Bath with Impeller Installed

Method A refers to the use of two samples of the fluid under

investigation and Method B to the procedure followed with corrosive liquids, which involved the application of Equation 1.

PROCEDURE

The method of introducing the sample into the evacuated calorimeter depended upon the nature of the material being added. If it was a pure substance and had a vapor pressure in excess of 1 pound per square inch at room temperature, it was introduced by distillation from a weighing bomb into the calorimeter assembly.
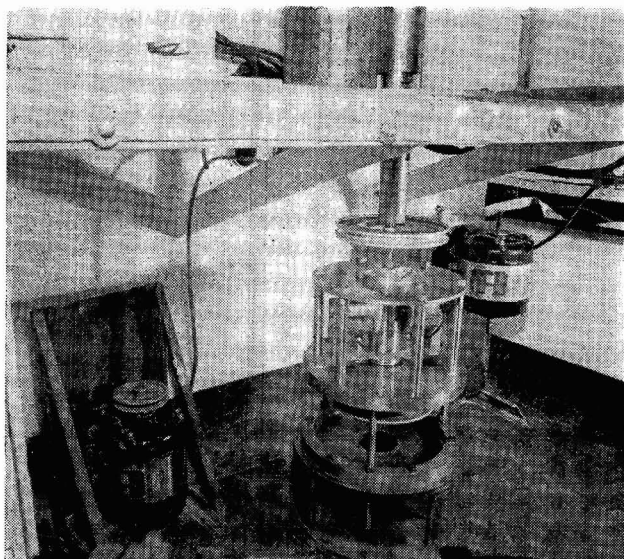

Figure 6. Agitator Drives

In this procedure, the valve below vessel N' (Figure 2) was closed after the material had been added, and the amount remaining in the connecting tubing was condensed into the weighing bomb by cooling the latter with liquid air. The quantity of material added by this procedure was ascertained by the change in weight of the weighing bomb. Two sets of measurements were usually made: one with the calorimeter only sufficiently full of liquid to permit circulation to be maintained; the other with the calorimeter as full of liquid as possible, considering the maximum specific volume of the saturated liquid at the highest temperature of the investigation. A minimum of 5% of gas space was desirable at all temperatures. The remainder of the procedure, except for calculations, was identical whether one or two samples were employed.
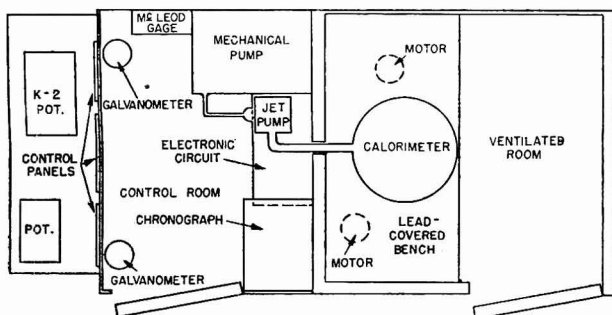

Figure 7. General Arrangement of Calorimeter and Control Equipment

The calorimeter and contents were brought to thermal and phase equilibrium by the use of the internal agitator, with the jacket maintained at the same temperature as the exterior of the calorimeter. In order to avoid excessive energy additions from mechanical agitation, the agitator was stopped before reaching final thermal equilibrium. The temperature of the calorimeter was then measured as a function of time. It did not remain

constant but drifted slightly in one direction or the other, the drift often amounting to less than 0.001° F. per minute. After the measurement had been taken, the agitator was started, and shortly thereafter energy was again added electrically. Efforts were made to ensure that the temperature of the vacuum jacket followed closely that of the calorimeter, and the temperature difference between the calorimeter and the jacket was checked frequently. After the temperature of the calorimeter was raised approximately 6° F., the addition of energy was discontinued, and the calorimeter was allowed to come to phase and approximate thermal equilibrium with the aid of mechanical agitation.
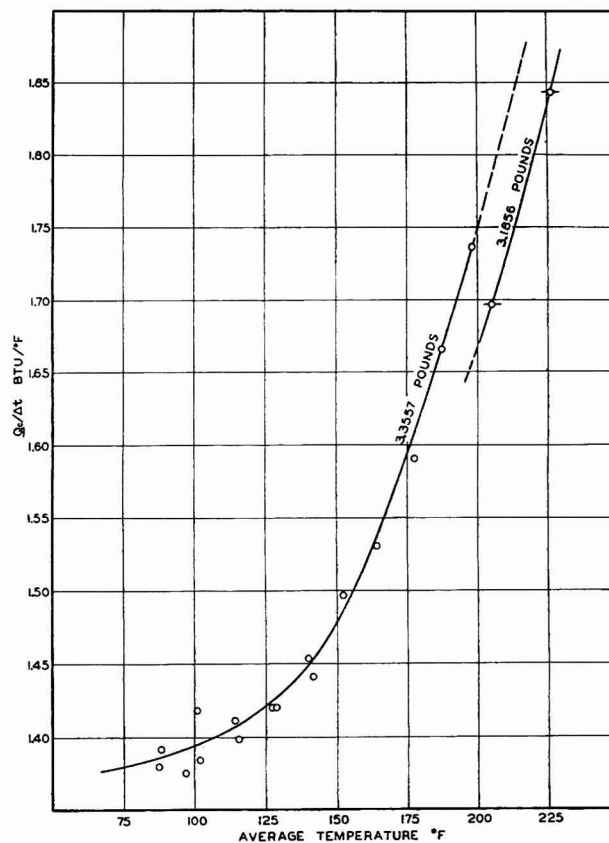

Figure 8. Experimental Calorimetric Data for Nitrogen Dioxide

Electrical addition of energy usually required approximately 10 minutes, and the re-establishment of thermal equilibrium, about an equal period of time. After thermal equilibrium was nearly attained again, the agitator was stopped and the temperature of the calorimeter was determined once more as a function of time. Corrections were made for the various factors discussed previously, utilizing Equation 1 or methods that have already been described (*12*).

HEAT CAPACITY OF NITROGEN DIOXIDE

As an example of the performance and application of the calorimeter, values obtained for the isobaric heat capacity of nitrogen dioxide are presented. In Figure 8 is shown the over-all heat capacity of the contents of the calorimeter as a function of temperature with samples of nitrogen dioxide weighing 3.35573 and 3.18563 pounds. However, because the sample weights were not widely different, Equation 1 was used to establish the heat capacity of this compound. In Table I are recorded the values of the heat capacity of the calorimeter and contents as well as the comparable values for the calorimeter alone. The resulting isochoric specific heat data for the heterogeneous nitrogen dioxide system are also included in this table. The values presented for the heat capacity of the calorimeter were obtained from an auxiliary set of measurements utilizing water as a working fluid.

Equation 1, in conjunction with available volumetric data for

nitrogen dioxide (4) and estimated heat capacities of the dew-point gas (3), was used to establish values of the isobaric heat capacity of the bubble-point liquid. In Table I and Figure 9 these values are shown for the experimentally determined points. Smoothed data for the isobaric heat capacity of nitrogen dioxide at bubble point are presented in Table II. The average deviation of the experimental points from a smooth curve in Figure 9 was 0.0024 B.t.u. per pound per ° F. The use of estimated values for the volumetric and phase behavior of the nitrogen dioxide may introduce 'uncertainties of as much as 2% in the isobaric heat capacity at the higher temperatures. The probable error in the value of the isochoric heat capacity of the heterogeneous system recorded in Table I is less than 0.5%.
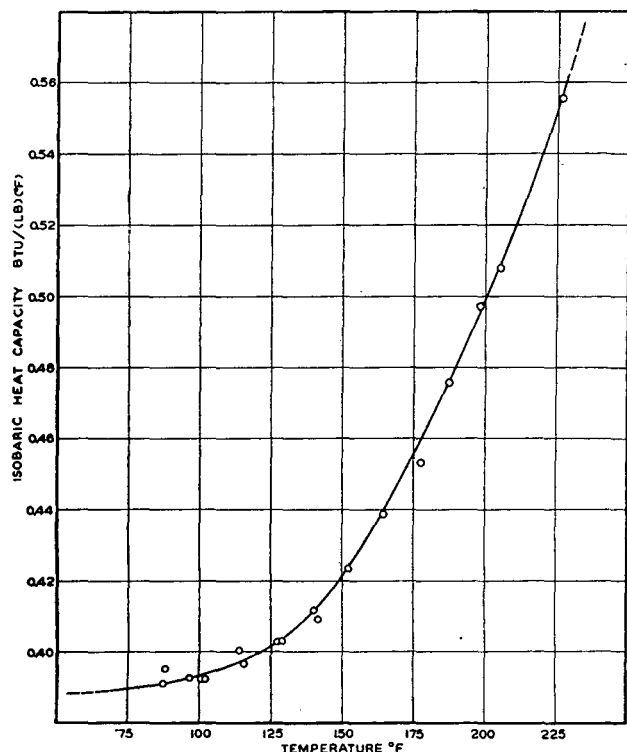

Figure 9. Isobaric Heat Capacity of Nitrogen Dioxide at Bubble Point

Table I. Experimental Calorimetric Measurements for Nitrogen Dioxide

Average Temp., ° F.	$\dfrac{Q}{T_2 - T_1}$, B.t.u./° F.	$\dfrac{Q_B{}^a}{T_2 - T_1}$, B.t.u./° F.	$\dfrac{Q_C}{T_2 - T_1}$, B.t.u./° F.	$\dfrac{Q_C}{m(T_2 - T_1)}$, B.t.u. (lb.)(° F.)	C_P, B.t.u. (lb.)(° F.)
88.20	1.9397	0.5479	1.3918	0.4053	0.3952
102.07	1.9368	0.5524	1.3844	0.4031	0.3925
115.70	1.9555	0.5569	1.3986	0.4072	0.3968
128.92	1.9812	0.5612	1.4200	0.4135	0.4031
141.83	2.0064	0.5654	1.4410	0.4197	0.4094
87.37	1.9275	0.5476	1.3799	0.4018	0.3917
100.89	1.9704	0.5520	1.4184	0.4031	0.3925
114.22	1.9676	0.5564	1.4112	0.4110	0.4004
127.32	1.9807	0.5607	1.4200	0.4135	0.4031
140.11	2.0182	0.5648	1.4534	0.4232	0.4120
152.49	2.0655	0.5689	1.4966	0.4338	0.4235
164.49	2.1136	0.5728	1.5408	0.4487	0.4390
177.92	2.1677	0.5772	1.5905	0.4631	0.4535
187.36	2.2463	0.5803	1.6660	0.4851	0.4760
198.21	2.3209	0.5838	1.7371	0.5058	0.4975
96.95	1.9261	0.5507	1.3754	0.4005	0.3926
205.26	2.2831	0.5861	1.6970	0.5199	0.5082
226.57	2.4370	0.5931	1.8439	0.5649	0.5558

$$ {}^a\ \frac{Q_B}{T_2 - T_1} = \frac{\Delta E_B}{T_2 - T_1} = \int_{\theta_1}^{\theta_2} \frac{dE_B}{dT}\frac{dT}{d\theta}\, d\theta. $$

Table II. Isobaric Heat Capacity of Nitrogen Dioxide at Bubble Point

Temp., ° F.	C_P, B.t.u.(lb.)(° F.)
70	0.3894
80	0.3903
90	0.3916
100	0.3934
110	0.3958
120	0.3994
130	0.4046
140	0.4113
150	0.4214
160	0.4342
170	0.4483
180	0.4638
190	0.4810
200	0.4992
210	0.5189
220	0.5410

NOMENCLATURE

C_P = isobaric heat capacity, B.t.u./(lb.)(° F.)
d = differential
E = specific internal energy, B.t.u./lb.
$\underline{E}$ = total internal energy, B.t.u.

m = weight, lb.
P = pressure, lb./sq. inch abs.
P'' = pressure in two-phase region, lb./sq. inch abs.
q = infinitesimal amount of heat, B.t.u.

$\underline{Q}$ = heat, B.t.u.

T = absolute temperature, ° R.
V = specific volume, cu. feet/lb.
θ = time, seconds
∂ = partial differential
Δ = finite increment
f = line integral

Subscripts

B = calorimeter
b = bubble-point gas
c = contents of calorimeter
d = dew-point gas
1 = initial
2 = final

LITERATURE CITED

(1) Bridgeman, O. C., *J. Am. Chem. Soc.*, **49**, 1174 (1927).
(2) Keenan, J. H., and Keyes, F. G., "Thermodynamic Properties of Steam," New York, John Wiley & Sons, 1936.
(3) McCollum, E. D., *J. Am. Chem. Soc.*, **49**, 28 (1927).
(4) Mittasch, A., Kuss, E., and Schleuter, H., *Z. anorg. allgem. Chem.*, **159**, 1 (1926).
(5) Osborne, N. S., Stimson, H. F., and Fiock, E. F., *Natl. Bur. Standards J. Research*, **5**, 411 (1930).
(6) Osborne, N. S., Stimson, H. F., and Ginnings, D. C., *J. Research Natl. Bur. Standards*, **23**, 197 (1939).
(7) Osborne, N. S., Stimson, H. F., and Ginnings, D. C., *Mech. Eng.*, **57**, 162 (1935).
(8) Osborne, N. S., and Van Dusen, M. S., *Bull. Bur. Standards*, **14**, 397 (1918–19).
(9) Sage, B. H., Evans, H. D., and Lacey, W. N., *Ind. Eng. Chem.*, **31**, 763 (1939).
(10) Sage, B. H., and Lacey, W. N., *Ibid.*, **27**, 1484 (1935).
(11) Sage, B. H., and Lacey, W. N., *Trans. Am. Inst. Mining Met. Engrs.*, **136**, 136 (1940).
(12) Schlinger, W. G., and Sage, B. H., *Ind. Eng. Chem.*, **41**, 1779 (1949).
(13) White, W. P., "The Modern Calorimeter," New York, Chemical Catalog Co., 1928.

Apparatus for Determining Gas Permeability of Carbon and Graphite

S. D. FULKERSON AND F. J. LAMBERT

Carbide and Carbon Chemicals Division, Union Carbide and Carbon Corporation, Y-12 Plant, Oak Ridge, Tenn.

The construction and operation of an apparatus for determining the gas permeability of carbon and graphite are described. The apparatus is built almost entirely of borosilicate glass, and is light, portable, and inexpensive. For all practical purposes results are of acceptable accuracy (about ±0.3%) and can be expressed either comparatively or in absolute units. Adoption as standard apparatus for measurement of working values of gas permeabilities is suggested.

CARTWRIGHT (1) has described an apparatus designed for measuring gas permeabilities, of thin plastic and other flexible sheet wrapping materials used in packaging food and drugs. Although the accuracy of this apparatus is good at permeabilities as low as 0.052 cc. per square meter per 24 hours per atmosphere (at normal temperature and pressure), it cannot be used for a more porous material such as carbon or graphite, because the units are entirely too small.

The principles involved in this apparatus are applicable to a more porous material, however, and by introducing certain modifications, carbon and graphite gas permeabilities can be satisfactorily determined. A recent need for such an instrument led to the development of the apparatus described below. It has proved so satisfactory that its adoption as a standard is suggested.

DESCRIPTION OF APPARATUS

The two principal parts of the apparatus, the upper and lower gas compartments, were improvised from two standard borosilicate glass flasks of 500- and 250-cc. volume, respectively, by fusing on the necessary attachments. The test specimen is clamped in place between these two compartments and sealed circumferentially to permit the passage of gas longitudinally only.

A third but smaller compartment made from a 50-cc. borosilicate glass bulb equipped with an inlet stopcock and outlet tube admits a measured amount of gas to the lower compartment after evacuation of both compartments. In this way exactly the same amount of gas under controlled conditions is admitted at the beginning of each test. The passage of the gas is then upward through the standard-size test specimen.

In order to know the absolute pressure at the start and finish of the test, both upper and lower compartments are equipped with a closed-end capillary mercury manometer tube. All essential parts of the main apparatus are pictured in the exploded view (Figure 1). The sliding millimeter scales for observing pressures within the upper and lower compartments are shown in the assembled view (Figure 2).

It is necessary to determine accurately the volume of one of the compartments; the authors chose the larger, upper compartment. It is well, however, to have accurate values for the volume of both compartments, because porosities may be determined from either volume and one can be used as a check for the other. There are two good ways of determining these volumes: by direct measurement and by gas-law calculation. The volume of the upper compartment, together with all side connections, was measured by first weighing it empty, then reweighing it after it had been completely filled with distilled water. Pure mercury could also be used. The volume is calculated from the weight of the liquid and its density at the temperature involved. If further accuracy is desired, a correction can be made for the weight of the air in the empty compartment.

The volumes of the compartments might be more conveniently calculated from the gas laws by sealing a glass plate over the mouth of the compartment with a nonpermeable wax, evacuating

the compartment, expanding into it a known quantity of gas from the metering bulb, and observing the increase in pressure. With either method, it is reasonable to assume that the error can be kept to less than ±1 cc. In a volume of 500 cc. this would amount to an error of 0.2%.

Perhaps the more vital parts essential to good results are the aluminum seal plate, the ground-glass joint at the mouth of the lower compartment, and the specimen itself. (For thin sections of a carbon sample added support is necessary to prevent cracking of the sample by the upward pressure. The aluminum seal plate with its notched knife-edge concentric rings is therefore necessary.) The dimensions must be accurately known and are therefore shown in detail in Figure 3. An error of 0.2% in their determinations cannot be tolerated and if the apparatus is to be duplicated, machining tolerances will have to be held to ±0.001 inch (0.025 mm.). This is not too difficult.
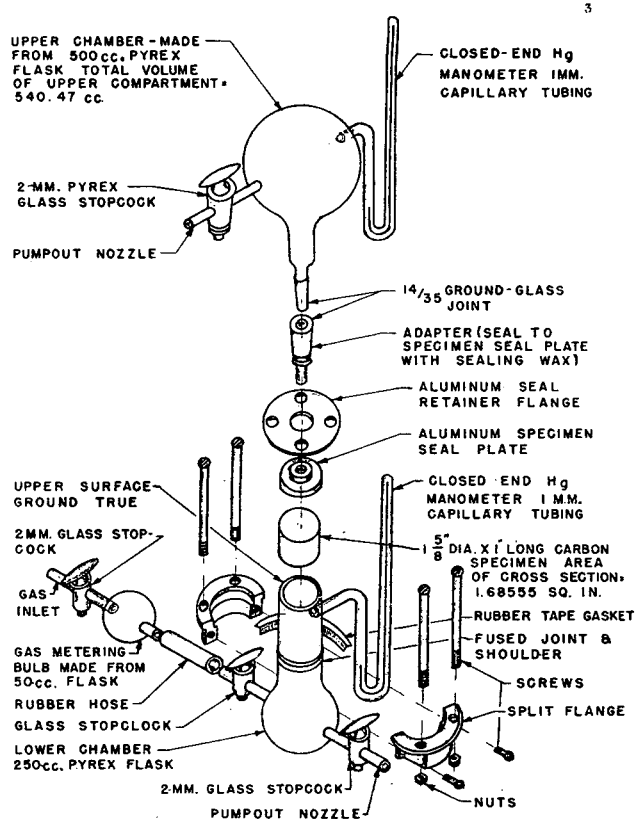

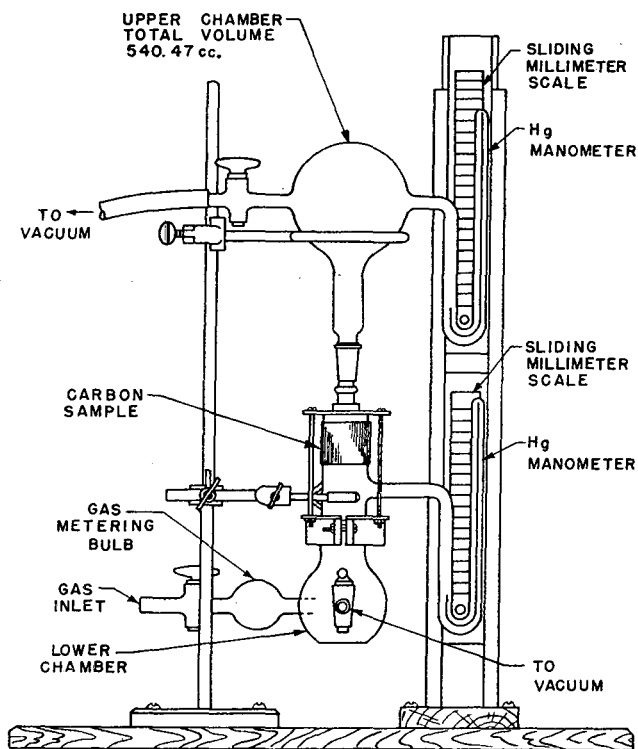**Figure 1. Exploded View of Gas Permeability Apparatus for Carbon**

Figure 2. Assembled Apparatus for Determining Gas Permeability of Carbon

scale. After two or three attempts, the sliding scale can be set so that little time will be lost in reading the initial pressure. It should be the same in each attempt, if the same quantity of gas is admitted each time and the apparatus is evacuated to the same starting pressure. At the same instant that the charge of metered gas is admitted, a stop watch is started to measure the time required for the pressure to reach a predetermined value in the upper compartment. This pressure value is arbitrary and should be reached in several hundred seconds by the passage of the gas upward through the specimen. The longer the time required, the more accurate the final pressure readings will be.

When various samples are compared, this predetermined test finishing pressure must always be the same. In this case, time in seconds is a direct indication of the degree of permeability; check runs on the same sample all agreed within 1 or 2 seconds. By averaging several runs further accuracy can be attained.

The permeability of a single sample can be determined and results reduced to any desired set of conditions. Should a standard unit of gas permeability of carbon and graphite be agreed upon, it would be possible to construct an apparatus, making such factors as cross-sectional area of the specimen, and perhaps volume of compartments, equal to unity. Calculations would then be greatly simplified.

CALCULATION OF RESULTS

In calculating the quantity of gas that passes through the sample in a given period of time, the initial and final pressure of one compartment, as well as the volume of that compartment must be known.
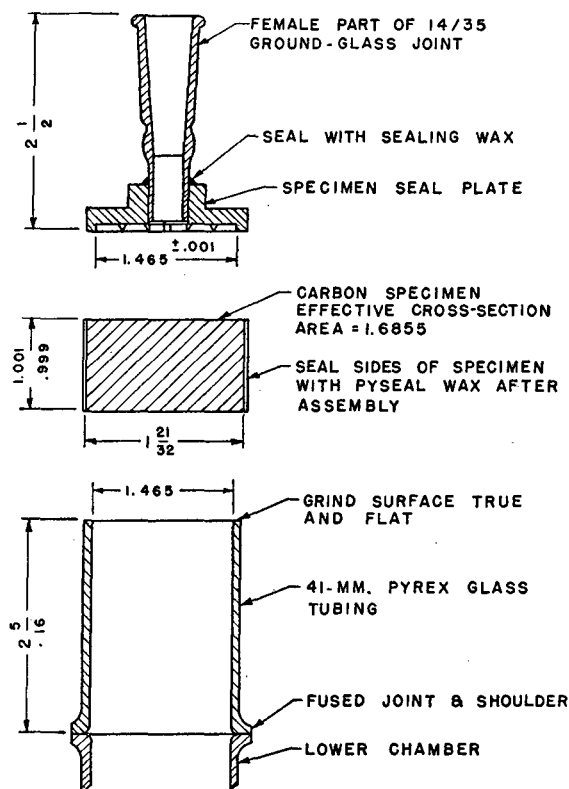
As shown in Figures 1 and 2, the apparatus is held together by means of aluminum flanges and machine screws. The lower flange is split in order to fasten it around the neck of the lower compartment just beneath the fused glass joint which provides a shoulder. A rubber tape gasket between the flange and glass protects the glass from breakage. The upper flange is in one piece, for in assembling, it readily passes over the female glass adapter which is sealed to the specimen seal plate with hard sealing wax.

The test specimen is made slightly larger in diameter than the outside diameter of either the seal plate or the ground-glass joint. In assembling, this provides a shoulder against which a nonpermeable wax such as Pyseal cement is applied. (This is a nonpermeable wax for sealing glass or metal, which can be obtained from the Fisher Scientific Company. It is satisfactory for vacuum or gas work below 70° C.) The specimen is held coaxially centered between the upper and lower chambers and sealed circumferentially against outside leaks by applying the Pyseal cement, using a small gas flame.

The 50-cc. gas metering bulb is secured to the lower compartment by means of a tight-fitting rubber tube and requires no further support.

The entire apparatus is held rigidly in a vertical position by means of a laboratory ring stand equipped with an upper ring and a lower ring stand clamp. To provide against any shifting of position of the main assembly or of the sliding scale assembly, both are fastened to a solid wood base by means of wood screws (see Figure 2).

OPERATION

After the apparatus has been assembled with the test specimen sealed in place, it is evacuated top and bottom by means of a laboratory mechanical pump to a pressure of about 100 microns, and the evacuation cocks are closed.

If after 10 minutes there has been no increase or decrease in pressure as observed from the manometers, it is assumed that not only is the system vacuum-tight but the adsorption of gas by the test specimen is negligible. (A 24-hour test was run to determine if there was enough gas adsorption by the specimen to produce a readable lowering of pressure. No change could be detected on either of the manometer scales.) At this point any desired gas can be measured into the 50-cc. bulb under any desired set of conditions and the inlet cock closed. The inlet cock to the lower compartment is opened and the measured charge of gas then assumes a volume equal to both the bulb and lower compartment. This volume has been previously determined, as discussed under description. The pressure is observed on the manometer

Figure 3. Specimen-Sealing Details of Apparatus

In the case of the described apparatus the volume of the upper compartment, V, was found to be 540.4 cc. If Q_1 is the quantity of gas initially in the upper compartment, it becomes increased by an amount ΔQ by the passage of a small amount of gas through the specimen, making a total of $Q_2 = (Q_1 + \Delta Q)$. The upper compartment then contains V cc. of gas at $(P_1 + \Delta P) = P_2$ mm. pressure, or a quantity of gas which can be expressed as P_2V mm.-cc. Therefore, ΔQ is easily determined from the expression $\Delta Q = (Q_2 - Q_1) = (P_2 - P_1)V$. Substituting the numerical value of V, the expression becomes simply

$$\Delta Q = 540.4 \Delta P \text{ mm.-cc.}$$

In this calculation, the expansion of ΔQ into the upper compartment has been considered as isothermal. Although it has been neither wholly isothermal nor adiabatic, the error introduced by disregarding temperature change is probably much less than the probable experimental errors incurred in volume determination and pressure readings. P can be read to the nearest 0.5 mm., and if the arbitrarily chosen test finishing pressure in the upper compartment be as much as 100 mm., the error in reading would never be greater than $\pm 0.5\%$.

SUMMARY

The apparatus described should be of value to anyone interested in the permeability of carbon and graphite. This apparatus is most adaptable for making comparisons between different grades or brands. By adapting an idea used in an oil viscometer where results are expressed in time units, involved calculations are avoided and these results are expressive of the relative permeabilities of two or more samples. The permeability of a single sample can be determined by making use of a few simple applications of the gas laws, and results can be converted to any desired set of conditions.

This apparatus has certain drawbacks. Results are not neces- sarily expressive of the average permeability of a large slab of carbon or graphite, for the slab has to be sampled at a local spot. Because sampling is destructive in nature, average sampling cannot always be tolerated. Finally, there are several sources of error introduced in its construction and operation.

In its favor the following points can be made: It is inexpensive to build, light in weight, and portable. Assembly is not difficult. Results are accurate for all practical purposes (about $\pm 0.3\%$), and can be expressed either comparatively or in absolute units. Its operation is simple, and its uses are not necessarily confined to carbon and graphite.

LITERATURE CITED

(1) Cartwright, L. C., ANAL. CHEM., 19, 393–6 (1947).

RECEIVED January 13, 1950. Based on work performed for the Atomic Energy Commission by Carbide and Carbon Chemicals Corporation, Oak Ridge, Tenn.

Determination of Traces of Mercury in Copper Alloys

Titration with Dithizone

W. L. MILLER AND L. E. WACHTER[1]

Material Laboratory, New York Naval Shipyard, Brooklyn, N. Y.

A method is described for determining as little as 0.04 p.p.m. of mercury in copper and its alloys. Mercury is reduced in sulfuric acid solution with stannous sulfate, distilled, and then titrated with dithizone (phenylazothionoformic acid phenylhydrazide, diphenylthiocarbazone). The method is not suitable for use with noble metal alloys.

IT IS known that traces of mercury may cause intergranular corrosion in copper alloys, but the analytical literature shows no method of suitable accuracy for determining 1 p.p.m. or less of mercury in such alloys. Fife (3) determined mercury in brass by microelectrodeposition; his method is suited to milligram rather than microgram amounts. The copper wire method of Stock and Zimmerman (7) and similar methods have been used for separating mercury from other metals. These methods become increasingly difficult and less reliable with reduced amounts of mercury and large concentrations of copper. Winkler (9), Reith and van Dijk (6), and others have determined microgram amounts of mercury by dithizone extraction with modifications to separate mercury from milligram amounts of copper. Several methods have appeared for determining mercury in the presence of copper and other interfering elements. In general, the methods have been limited to the presence of either several micrograms of mercury or a relatively small amount of copper.

Kozelka (4) described a method of separating mercury by distillation in a stream of chlorine from concentrated sulfuric acid containing 1.0 gram of copper sulfate. Although this method was an improvement over hydrochloric acid distillations (2, 8), it was not found suitable for 1- to 5-gram samples of copper and brass. When sufficient temperature was maintained to distill a large percentage of the mercury salts, interfering amounts of copper were carried into the distillate. Attempts to prevent this by use of a spray trap resulted in very low recoveries of mercury. The method was further complicated by a tendency of copper salts to plug the chlorine intake tube during distillation. While satisfactory results were not obtained, because of the concentration of copper salts, Kozelka's method appears practical for copper alloys when mercury contents permit the use of less than 1-gram samples.

Further experiments showed that up to 15 micrograms of mercury are easily distilled as metal from a dilute sulfuric acid medium. Mercury is preferentially reduced to metal with stannous sulfate, and is distilled with steam generated within the distilling flask. The method is particularly suited to small amounts of mercury, with little tendency to coagulation and a rapid transition to the vapor phase. The distilled mercury is trapped in a sulfuric acid–permanganate solution similar to that used by Kuzyatina (5). Stannous sulfate is used instead of stannous chloride because large amounts of copper, in the presence of chloride ions, were found to cause poor recovery of mercury. Large excesses of stannous chloride did not prevent this.

The sample is dissolved slowly in sulfuric acid and hydrogen peroxide. A residue of mercury may remain, but this is dissolved by additional peroxide and gradual heating. Excess peroxide

is then decomposed by boiling. This method eliminates the necessity of heating to sulfuric acid fumes with consequent volatilization losses of mercury salts. Attempts were made to hasten

solution by heating under a reflux condenser and also by dissolving in the assembled distillation apparatus. Losses of mercury occurred, probably due to volatilization of metallic mercury, oxidation in the condensers, and precipitation on the side walls.

Because of the comparatively clean separation of mercury by reduction-distillation, a direct titration with dithizone is convenient and has advantages for very small amounts. It is unnecessary to purify the dithizone, and as little as 0.2 microgram of mercury (Hg^{++}) will give a characteristic orange end point. A chloroform solution of dithizone is used for titration to prevent interference from possible traces of copper. As shown by Barnes (1), a chloroform solution of dithizone is considerably more selective for mercury than a carbon tetrachloride solution. The present authors have found that the presence of one-tenth volume of carbon tetrachloride has little, if any, effect and prefer this solvent for storage purposes. Although the possibility of errors from metals other than mercury entering the distillate cannot be ignored, no such errors have been detected by the authors.

The best value for the dithizone solution is obtained by standardizing immediately following the titration. The titer will vary somewhat with the amount of mercury and the manner of titration. Formation of varying amounts of enol dithizonate as well as the predominant keto compound evidently causes this. Errors are minimized by following approximately the same additions in titrating both sample and standard, particularly when several micrograms are determined.

Excess permanganate in the distillate is reduced immediately before titration by a slight excess of sulfurous acid. A large excess of sulfurous acid was found to cause errors due to reduction of mercury to metal. Although this reduction does not occur in hydrochloric acid solutions, it may be readily observed with macro amounts of mercury salts in sulfuric acid solutions.

Although the method was developed for copper and copper alloys, it should be useful for micro amounts of mercury in various materials. It is not suitable for materials containing noble metals that are reduced to metal by stannous sulfate. However, tests made with added silver salts showed no interference with 12 mg. of silver; 25 mg. of silver gave a pronounced precipitate with about 80% recovery of mercury. It may be assumed that unless purposely added, noble metals are not present in interfering amounts in copper or its alloys. Insoluble matter will adsorb mercury, with a tendency to low results.

APPARATUS AND REAGENTS

An all-glass apparatus is required, as shown in Figure 1. A small amount of lubricant may be used on stopcock A, but only water is used to lubricate joint B. All parts are rinsed with hot 10% sodium hydroxide solution after use to prevent accumulation of scale. If visible scale forms in tube E and cannot be removed, the tube should be replaced.
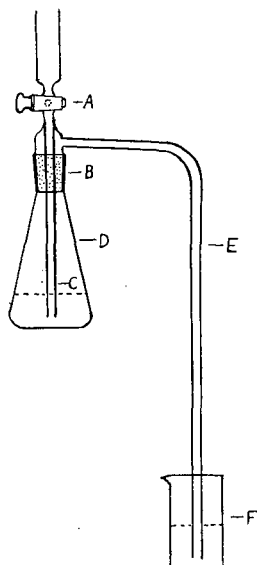

Figure 1. Apparatus

A. **Funnel stopcock**
B. **24/40 ground-glass joint**
C. **Funnel stem**
D. **500-ml.   Erlenmeyer flask**
E. **7 mm. × 60 cm. glass tube**
F. **200-ml. tall-form beaker**

Sulfuric acid, concentrated, c.p.
Hydrogen peroxide, 30%, c.p.
Copper sulfate pentahydrate tested mercury-free.
Stannous Sulfate Solution. Dissolve 50 grams of stannous sulfate in 500 ml. of distilled water, add 1 drop of concentrated sulfuric acid, shake, and let settle in a well stoppered bottle. Decant clear solution as needed.
Permanganate–Sulfuric Acid Solution. Add 25 ml. of concentrated sulfuric acid to redistilled water, cool, and adjust to 250 ml. Add 250 ml. of 0.2 N potassium permanganate (in redistilled water), and mix.
Sulfurous Acid, redistilled water saturated with sulfur dioxide. Keep well stoppered and renew frequently.
Dithizone Stock Solution. Dissolve 12.0 mg. of dithizone in 500 ml. of redistilled carbon tetrachloride. Keep in a dark glass bottle stored in the dark.
Dithizone Titrating Solution. Dilute 1 volume of the stock solution to 10 volumes with redistilled chloroform as needed. Avoid exposure to strong light. One milliliter equals approximately 1 microgram of mercury.
Mercury Standard Solution. Dissolve approximately 250 mg. of mercury in 30 ml. of concentrated nitric acid and dilute with distilled water to give 0.5 mg. per ml. Dilute further with distilled water as needed to give 1 or 5 micrograms of mercury per ml. Renew the stronger solution within 1 week and the weaker solution daily.

PROCEDURE

Add 50 ml. of distilled water and 11 ml. of concentrated sulfuric acid to the 500-ml. distilling flask, separated from the apparatus. Chill in ice water. Add 1 or 5 grams of sample chips containing not over 15 micrograms of mercury (1 gram only for high-lead or high-tin bronzes). Add 15 ml. of 30% hydrogen peroxide, cover with a small cover glass, and let stand in cold water. If pronounced frothing occurs, chill with ice to slow the reaction. For 5-gram samples, add 10 ml. of distilled water and 15 ml. more of 30% hydrogen peroxide after the initial reaction. Occasional samples may need further peroxide additions to ensure solution. Let the flask stand overnight in water at room temperature. The samples should then show only a fine white or gray residue. Add 5 ml. more of 30% hydrogen peroxide and heat very gradually to boiling. Gas should evolve slowly without frothing. Boil gently until signs of decomposing peroxide have disappeared, then uncover and continue boiling for 15 minutes more. Adjust the volume to approximately 110 ml. for clear solutions, or 130 ml. for turbid solutions (usually with tin or lead).
Add 50 ml. of permanganate–sulfuric acid solution to the receiving beaker, and assemble the distilling apparatus with the receiver immersed in a pan of cool water. Add 20 ml. of stannous sulfate solution slowly through the funnel and rinse with 5

Table I. Recovery of Mercury from Copper-Base Metals

Material[a]	Hg Added γ	Hg Recovered γ	Error γ
Brass (70 Cu:30 Zn), 5 grams	1.0	1.0	0.0
	1.0	1.1	+0.1
	1.0	0.9	−0.1
	1.0	0.8	−0.2
Brass (70 Cu:30 Zn), 1 gram	10.0	10.0	0.0
	10.0	9.4	−0.6
	10.0	10.4	+0.4
	15.0	14.4	−0.6
	15.0	14.9	−0.1
	15.0	14.0	−1.0
	15.0	14.0	−1.0
Bronze (88 Cu:9 Sn:3 Zn), 1 gram	1.0	0.7	−0.3
	1.0	0.7	−0.3
	1.0	0.9	−0.1
	5.0	5.0	0.0
	5.0	4.5	−0.5
	5.0	4.8	−0.2
Bronze (75 Cu:19 Pb: 6 Sn)	10.0	8.5	−1.5
	10.0	8.4	−1.6
	10.0	8.4	−1.6
	10.0	9.0	−1.0
Copper, 5 grams	0.2	0.3	+0.1
	0.2	0.2	0.0
	0.2	0.2	0.0
	0.2	0.2	0.0
	5.0	4.6	−0.4
	5.0	4.9	−0.1
	5.0	4.8	−0.2

[a] No mercury, other than reagent blanks, was detected with 5-gram samples of original materials.

ml. of distilled water. Heat to boiling and close the stopcock when boiling starts. The boiling should be fairly rapid, so that the condensate is near boiling, but without steam bubbles rising from the receiver. Add cool tap water to the cooling pan as the distillate level rises. Distill 60 ml. from clear sample solutions or 80 ml. from turbid solutions. (Slight turbidity from reaction of added stannous sulfate is ignored.) Stop the heating, and as the pressure drops, open the stopcock to prevent sucking back.

Cool the distillate to room temperature. Add saturated sulfurous acid slowly with stirring until the permanganate is decolorized, then add 5 drops in excess. Transfer the solution to a 250-ml. separatory funnel. From a 10-ml. buret add approximately one half the estimated required amount of dithizone titrating solution. Shake vigorously for 20 seconds, let settle 1 to 2 minutes, and drain off the chloroform layer. Repeat, using 0.5-ml. or 1.0-ml. additions of dithizone until a definite orange colored chloroform layer is no longer produced. Then shake 20 seconds more and let settle 2 to 3 minutes. If a mixed color is obtained, the amount of reacted dithizone may be estimated. Continue the titration with 0.5-ml. or 0.25-ml. additions until a green chloroform layer confirms an end point within the last previous addition.

Avoid exposure to strong light and keep the buret stoppered between additions. Record each addition. If abnormal amounts of copper are present, a purple-red color following the mercury titration may prevent a satisfactory end point, in which case the determination should be repeated.

Add standard mercury solution, containing approximately the amount of mercury titrated, to 50 ml. of permanganate-sulfuric acid solution. Add 60 ml. of redistilled water, then treat and titrate as above, following essentially the same additions as with the sample. This gives an accurate titer for the dithizone solution. Where best precision is not required, mercury may be added to the titrated sample for standardization.

Modification for Mercury Less Than 1.0 Microgram. Follow the above procedure, but prior to titration saturate the sample solution by shaking with a few milliliters of chloroform. Let settle well and drain off excess chloroform. Titrate with 0.25-ml. additions of dithizone. Increase the above shaking periods by 20 seconds and the settling periods by 1 minute.

Determine the mercury blank in all reagents by following the above procedure, using 1 gram of tested copper sulfate in place of the sample. (Copper is used to decompose hydrogen peroxide by catalysis.) The copper sulfate may be tested by the difference between blank runs with and without copper sulfate, excluding the use of peroxide.

RESULTS

Table I illustrates recovery of mercury from representative copper-base metals. Standard mercury solution was added to mercury-free samples and allowed to stand various periods before dissolving. Correction was made for reagent blanks ranging from 0.1 to 0.3 microgram. Of the reagents used, only the hydrogen peroxide gave measurable amounts of mercury.

Approximately 95% recovery of added mercury was obtained from copper and brass. Somewhat lower recoveries were obtained from the bronze samples, owing to adsorption of mercury by lead sulfate and metastannic acid.

While the method provides for detection of interfering amounts of copper in the distillate, such interference would probably be rare. In several distillations made from copper sulfate solutions containing 5 grams of copper in the absence of added mercury or peroxide reagent, the authors obtained zero titrations in every instance. Addition of 0.1 microgram of mercury to the copper solution was sufficient to produce a mixed color with 0.25 ml. of titrating solution. The method is therefore well suited for the determination of minute amounts of mercury and it compares favorably with other methods used with less difficult base materials.

LITERATURE CITED

(1) Barnes, H., *Analyst*, 72, 469–72 (1947).
(2) Fenimore, E. P., and Wagner, E. C., *J. Am. Chem. Soc.*, 53, 2468–75 (1931).
(3) Fife, J. G., *Analyst*, 63, 650–1 (1938).
(4) Kozelka, F. L., ANAL. CHEM., 19, 494–6 (1947).
(5) Kuzyatina, N. S., *Zavodskaya Lab.*, 8, 174–7 (1939).
(6) Reith, J. F., and van Dijk, C. P., *Chem. Weekblad*, 37, 186–93 (1940).
(7) Stock, A., and Zimmerman, W., *Z. angew. Chem.*, 41, 546–8 (1928).
(8) Strecker, W., and Conradt, K., *Ber.*, 53, 2113–27 (1920).
(9) Winkler, W. O., *J. Assoc. Offic. Agr. Chemists*, 18, 638–44 (1935).

Determination of Small Amounts of Vanadium in Steel by Photometric Titration

ROBERT F. GODDU AND DAVID N. HUME

Massachusetts Institute of Technology, Cambridge 39, Mass.

The ferrous sulfate-persulfate method for the determination of vanadium has been improved by a photometric determination of the end point. This results in an increase of the accuracy and precision as shown by analysis of standard steel samples. High results are obtained if the ratio of chromium to vanadium is greater than 10 to 1. If tungsten is separated, other elements common in steel do not interfere.

VANADIUM in steel is customarily determined volumetrically, by either reduction with ferrous ion or oxidation with potassium permanganate (7). It was felt that the permanganate titration in the popular ferrous sulfate-persulfate method (1, 7) could be improved for greater accuracy and for use in colored solutions by determining the end point photometrically.

Somiya (11) stated that photometric permanganate titration was possible, but gave no details. In a photometric titration, a plot is made of the absorbancy of the solution against increments of titrant added. The titration curve will generally consist of two straight lines intersecting at the equivalence point. By use of monochromatic light, it is possible to measure many color changes without interference from colored constituents already present in the solution. With photoelectric measurement of light intensity, the sensitivity of the method is much greater than that of direct visual observation.

The precision of a photometric titration is considerably greater than that of direct colorimetric measurement because the end point is determined by the intersection of two curves, each of which is determined by the average of several points. It is usually possible to choose conditions such that a relatively large error in the individual photometric measurements results in only
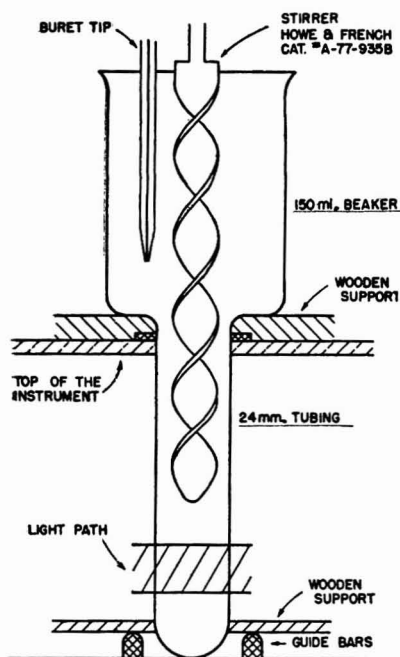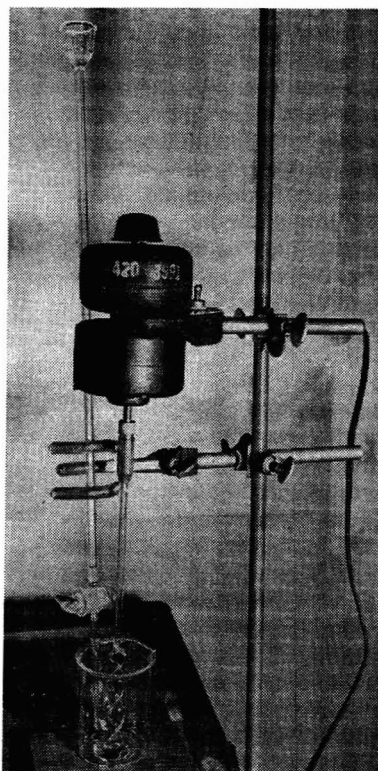
Figure 1. Cross-Sectional View of Titration Cell

Figure 2. Titration Cell

Figure 3. Absorption Spectra in Beckman DU Spectrophotometer

a small end-point error. Because measurements taken in the vicinity of the equivalence point have no especial significance the method, like amperometric and conductometric titration, is particularly useful for reactions which are appreciably incomplete at the equivalence point. The linear dependence of absorbancy upon concentration results in a sharper end point than would be obtained in a potentiometric titration, because potential is a logarithmic function of ion concentration. By proper choice of wave length, path length, and instrument sensitivity, the method is applicable over a wide range of concentration of colored constituents.

Several applications of the photometric titration principle have been described in the literature. A review of the progress up to 1943 can be found in the paper of Osburn, Elliot, and Martin (8). Work published since then is included in the bibliography of this paper (2, 3, 9, 10, 12). Hitherto, photometric titrations have been performed with homemade apparatus or commercial photometers which are not readily adaptable to general use. One purpose of the present investigation was, therefore, to devise a simple and relatively inexpensive means of adapting a common, commercially available spectrophotometer to this type of measurement.

APPARATUS

The instrument chosen for this work was the Coleman Model 14 spectrophotometer. To accommodate a large volume of solution and still have the sample in the light path, the cell shown in Figures 1 and 2 was devised, similar in principle to that used by Osburn, Elliot, and Martin (8). Efficient mixing is obtained within 1 minute by using a rotary stirring motor with the end of the stirring blade just above the light path (Figure 1).

OPERATING TECHNIQUE

The spectrophotometer is turned on and allowed to warm up until the position of the galvanometer is steady, after which the sample is transferred to the cell already in the light path and the monochromator is set to the desired wave length (in this case, 525 mμ). The stirrer is turned on and when the solution is homogeneous, the galvanometer is adjusted to 0.000 optical density or to some other convenient value before the titration is begun.
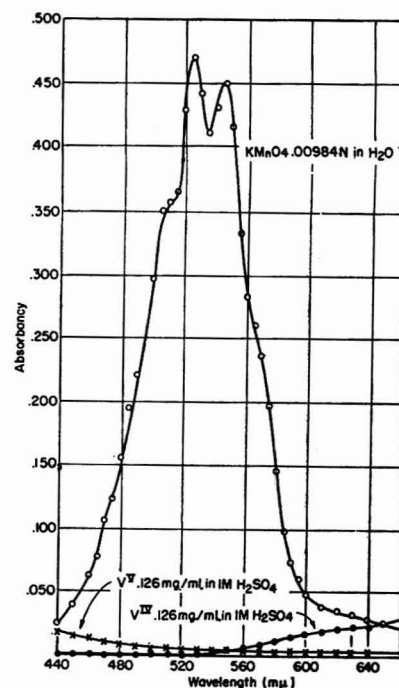
Aliquots are added from a 5-ml. buret whose tip is below the level of the solution (no appreciable diffusion was found from the Kimble Exax 5-ml. burets used). When the galvanometer reading reaches a constant value, it is noted and another aliquot is added. Points near the end point may fluctuate and be off the curve, owing to the incompleteness of the reaction. These points close to the equivalence point are of no special significance and can be disregarded. The best straight lines are drawn between the points taken well before and after the equivalence point; the intersection is taken as the end point. If relatively large volumes of titrant are added after the end point, a volume correction of the type used in amperometric and conductometric titrations may be necessary.

Because we are interested only in relative shifts in absorbancy, there is no need to adjust the instrument to read absorbancy against a reference solution, providing the instrument is stable. The galvanometer of the Coleman was found not to drift appreciably after the instrument had been allowed to warm up for 15 minutes. To eliminate volume corrections, the reagent was added in concentrated form from a 5-ml. buret. A titration requires 10 to 15 minutes, depending on the number of points taken.

MATERIALS

Standard Vanadium Solution. The standard vanadyl solution was prepared from Amend's c.p. ammonium metavanadate (found spectrographically to contain only traces of iron) by sulfur dioxide reduction (7). It was standardized potentiometrically with standard permanganate (5). All the reagents used in these studies were the purest obtainable.

EXPERIMENTAL

Inspection of the absorption curves of the ions involved (Figure 3) showed that at 525 mμ the permanganate absorption would be measured without interference from vanadium. Preliminary titrations were done with pure vanadium solutions to determine the applicability of the method. These titrations of the pure vanadyl solutions in 1 M sulfuric acid gave good curves with sharp end points, such as that in Figure 4. For concentrations of vanadium down to 10 micrograms per ml., the reaction was rapid and accuracy of 1% or better was obtainable. At lower concentrations the reaction was slow, resulting in an appreciable

increase of absorbancy during the titration, and thus giving a high result. Extrapolation of the permanganate absorption line to the base line still gave 1% accuracy and precision with concentrations as low as 5 micrograms per ml. This extrapolation is valid because no change in absorbancy occurs before the equivalence point due to the oxidation of vanadyl ion.

The effect of other elements commonly used to alloy steels was tested by titrating pure vanadium solutions with these constituents added. The only interferences found were tungsten, which must be filtered off as tungstic acid, and chromium, when present to a much greater extent than the vanadium. By titrating solutions with different ratios of chromium to vanadium, it was found that unless this ratio is larger than 10 or 15 to 1, the error incurred is no greater than 1 or 2%—i.e., within the accuracy of the method. An error due to chromium was also noted by Hamner in his original paper on the persulfate method (1). The authors found that the oxidation of chromium with permanganate takes place to a very small extent, if at all, in the absence of vanadium. The extent of the oxidation increases with actual amount of vanadium as well as with the chromium-vanadium ratio. Presumably, the chromium might be eliminated from high-chromium samples by volatilization from perchloric acid–hydrochloric acid medium (4).

STUDY OF PERSULFATE METHOD

In the titration of pure vanadyl solutions by the ferrous sulfate–persulfate method of Lundell, Hoffman, and Bright (7), the results with milligram amounts of vanadium were consistently found to be lower than those obtained by direct titration. The results were dependent on both the amount of excess persulfate and the time elapsed between its addition and the titration. A systematic study of the conditions necessary for an accurate titration revealed that both of these variables must be carefully controlled. The amounts of persulfate added must be only slightly more than that required to oxidize the excess ferrous ion present.
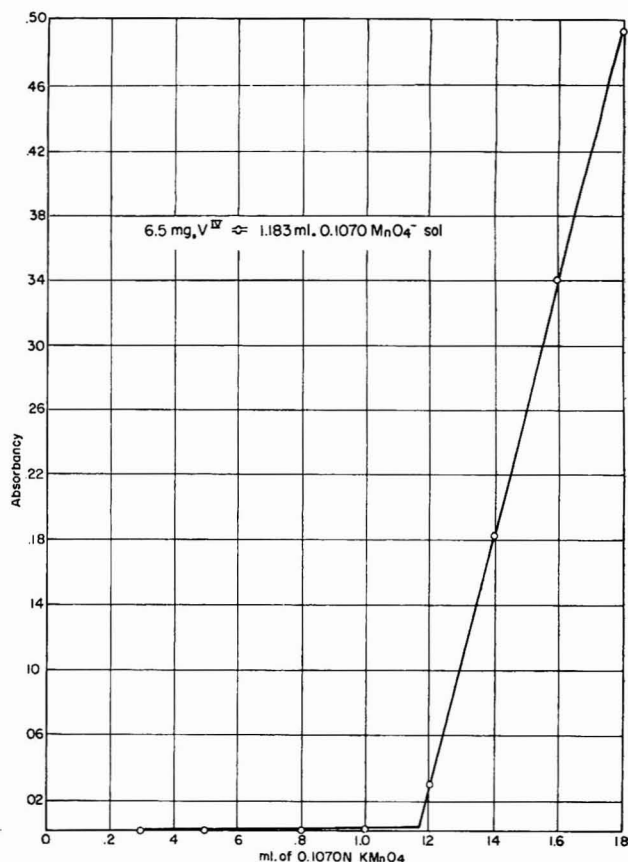

Figure 4. Titration of Vanadium

6.5 mg. of vanadium with 0.1070 N potassium permanganate in 100 ml. of 1 M sulfuric acid at 525 mμ on Coleman Model 14 spectrophotometer

A known excess of ferrous ion can be used only if some means is available for determining when enough has been added to complete the reduction of any oxidized chromium, manganese, or vanadium. This is done most advantageously by detecting the first slight excess with ferricyanide on a spot plate, then adding a known amount.

APPLICATION TO STEEL SAMPLES

The following procedures were developed from standard methods (6, 7) in order to give the desired conditions for a photometric determination. The usual solution procedures utilizing sulfuric and nitric acids gave satisfactory results on standard steels containing significant amounts of vanadium, but showed an appreciable blank on carbon steels very low in vanadium. The use of perchloric acid eliminated this difficulty, presumably because the carbon present was completely oxidized when the mixture was fumed. The addition of hydrochloric acid was also found necessary in the analysis of tungsten steels.

Procedure for Analysis of Steel Samples. Into a 150-ml. beaker a sample containing 5 to 15 mg. of vanadium is weighed if 0.1 N permanganate is to be used (1 ml. of 0.1 N permanganate = 5.1 mg. of vanadium), 10 ml. of water and 12 ml. of 60% perchloric acid are added, and the sample is heated on a hot plate until it is decomposed. If tungsten is present, 2 ml. of 1 to 1 hydrochloric acid are added. The sample is boiled over a low flame until the fuming perchloric acid has oxidized the chromium and manganese, as indicated by their characteristic orange or red colors. At this point all the carbon has been oxidized.

If tungsten is present, the precipitate of tungstic acid must be filtered off. The solution is diluted to about 30 ml. with water, heated, and filtered through dense filter paper—e.g., S. & S. No. 589 Blue Ribbon—into another 150-ml. beaker or the titration

Table I.   Analyses of Standard Samples

N.B.S. Standard Sample and Certificate Values	No. of Determinations	Vanadium Found, %	Standard Deviation, %
Cr-V steel 30d V 0.190, C 0.363, Mn 0.786, P 0.031, S 0.031, Si 0.286, Cu 0.092, Ni 0.150, Cr 1.15, Mo 0.034	6	0.190	0.002
Mo-W-Cr-V steel 132 V 1.64, C 0.803, Mn 0.252, P 0.027, S 0.004, Si 0.239, Cu 0.149, Ni 0.094, Cr 4.11, Mo 7.07, W 6.29	5	1.63	0.01
Mo-W-Cr-V steel 134 V 1.13, C 0.810, Mn 0.155, P 0.016, S 0.006, Si 0.0323, Cu 0.114, Ni 0.077, Cr 3.37, Mo 8.68, W 1.82	5	1.12	0.01
Mo-W-Cr-V-Co steel 153 V 2.04, C 0.864, Mn 0.219, P 0.025, S 0.008, Si 0.187, Cu 0.099, Ni 0.107, Cr 4.14, Mo 8.38, W 1.58, Co 8.45	5	2.02	0.02
Ferrotitanium 116a V 0.33, Ti 25.06, C 0.023, P 0.18, Si 3.12, Cr 0.23, Al 3.25	5	0.331	0.007

Table II.   Typical Results on Chrome-Vanadium Steel 30d

(Bureau of Standards value, V = 0.190%)

No.	Sample, Grams	Vanadium Found, Mg.	Vanadium Found, %
1	0.997	1.91	0.192
2	1.014	1.94	0.191
3	1.013	1.89	0.186
4	1.023	1.94	0.190
5	1.169	2.22	0.190
6	1.149	2.19	0.191

Mean = 0.190 ± 0.001
Standard deviation (σ) = 0.002

cell. The precipitate should be washed with hot 0.5% per-chloric acid to aid filtration.

With regular steels the sample is cooled, the spattered drops are rinsed down, and the solution is diluted to about 80 ml. A little colloidal silica, if present, does no harm.

Both tungsten and other steels are treated similarly from here on.

The sample solution is transferred to the titration cell with 0.5% perchloric acid, and 3 ml. of 85% phosphoric acid and then 0.1 N ferrous sulfate solution (in 0.5% perchloric acid) are added until a slight excess is shown by ferricyanide on a spot plate. Then 5.0 ml. of the ferrous solution in excess are added.

The instrument is adjusted, as previously described, to a wave length of 525 mμ, and 2.5 ml. of freshly prepared ammonium per-sulfate solution (15 grams per 100 ml.) are added. After 1 minute, the solution is titrated with standard permanganate solution. If 0.1 N permanganate is used, 0.1-ml. increments are most convenient, while with 0.02 N permanganate 0.4-ml. portions are suitable.

Procedure for Ferrotitanium. A 0.5-gram sample containing 1 to 2 mg. of vanadium is weighed out into a 300-ml. Kjeldahl flask, and 25 ml. of water and 10 ml. each of 1 to 1 sulfuric, nitric, and hydrochloric acids are added. The sample is boiled gently over a flame until decomposition is complete, more nitric acid, hydrochloric acid, or water being added if necessary. Then the solution is boiled down twice to fumes of sulfur trioxide and cooled, 50 ml. of water are added, and the solution is filtered through medium porosity paper. The flask and precipitate are washed with 0.1 N sulfuric acid; finally, the filtrate is diluted to 100 ml. with water and transferred to the titration cell. The procedure used with steel is then followed.

DISCUSSION OF RESULTS

Tables I and II show that the accuracy of this method equals that of the methods used by the cooperating analysts in the analysis of the National Bureau of Standards standard samples. This procedure allows the convenient persulfate method to equal or surpass the accuracy of other existing methods for determining milligram amounts of vanadium. In addition to the determination of vanadium in steel and ferrous alloys, this general procedure should be applicable to other systems where an accurate determination of small amounts of vanadium is required.

LITERATURE CITED

(1) Hamner, H. L., *Met. Chem. Eng.*, 17, 206 (1917).
(2) Hindeman, J. C., Magnusson, L. B., and La Chapelle, T. J., *J. Am. Chem. Soc.*, 71, 687 (1949).
(3) Hirano, S., *J. Soc. Chem. Ind. (Japan)*, 46, 233 (1943).
(4) Hoffman, J. I., and Lundell, G. E. F., *J. Research Natl. Bur. Standards*, 22, 465 (1939).
(5) Kolthoff, I. M., and Furman, N. H., "Potentiometric Titrations," 2nd ed., New York, John Wiley & Sons, 1931.
(6) Lundell, G. E. F., and Hoffman, J. I., "Outlines of Methods of Chemical Analysis," New York, John Wiley & Sons, 1938.
(7) Lundell, G. E. F., Hoffman, J. I., and Bright, H. A., "Chemical Analysis of Iron and Steel," New York, John Wiley & Sons, 1931.
(8) Osburn, R. H., Elliot, J. H., and Martin, A. F., IND. ENG. CHEM., ANAL. ED., 15, 642 (1943).
(9) Raeder, M. G., *Chem. Zentr.*, 1943, II, 11,982.
(10) Reznik, B. E., and Fedorova, G. P., *Zhur. Anal. Khim.*, 3, 92 (1948).
(11) Somiya, T., and Nakamura, Y., *J. Soc. Chem. Ind. (Japan)*, Supplemental Binding, 38, 262B (1935).
(12) Zamecnik, P. C., Levin, G. I., and Bergman, M. J., *J. Biol. Chem.*, 158, 537 (1945).

Determination of Small Amounts of Chromium in Human Blood, Tissues, and Urine

Colorimetric Method

PAUL F. URONE AND HANNS K. ANDERS

Division of Industrial Hygiene, Ohio Department of Health, Columbus, Ohio

A rapid, colorimetric method for the determination of small amounts of chromium in human blood, tissues, and urine is described. The method is sensitive to 0.005 microgram of chromium per milliliter of final solution. Samples are ashed in borosilicate glass beakers, using a combination of wet- and dry-ashing methods. The blood and tissue samples are oxidized by bromine in alkaline solution. Oxidation of the urine samples is accomplished by sodium bismuthate in acid solution. Diphenylcarbazide is used as the color reagent. A clear red-violet color is obtained which can be measured colorimetrically or spectrophotometrically. Comparative standardization curves and typical analyses are given.

IN A recent industrial cancer investigation it became necessary to determine small amounts of chromium in human blood, urine, and autopsy specimens. As the number of samples to be run was large, it was necessary to develop new, sensitive, and more rapid techniques than those reported in the literature.

The best methods for estimating small amounts of chromium are colorimetric methods using diphenylcarbazide (3), which gives a clear red-violet color with minute amounts of chromium (29). Diphenylcarbazide was first used by Moulin (23). It has since been used extensively, and a large number of the influencing factors and interferences have been studied and reported (5, 9, 10, 27, 29, 37, 39). The method is nearly specific for chromium; hexavalent molybdenum is the only element giving a similar, but much less sensitive, violet color in acid solution (29). Mercury, copper, cadmium, silver, lead, nickel, cobalt, manganese, magnesium, zinc, vanadium, and iron also give colors under various conditions (11, 37). However, under the analytical conditions used for determining chromium, most of these metals either react with low sensitivity or not at all (10, 27).

Only a few methods have been given in the literature for the determination of chromium in physiological specimens, although many methods appear for leather, which often contains large amounts of chromium introduced during the tanning process. Investigators have reported quantitative methods for the deter-

mination of chromium in human tumors (6, 7), animal tissues (2), tissue ash (26), plant ash (36), milk (35), fats (31), toxicological specimens (17), vegetation (28), animal feces (21), and sewage (10, 32).

ASHING

A majority of the investigators cited have reported dry-ashing methods for the physiological specimens. Usually, platinum ware and muffle furnaces at a temperature of approximately 500° C. were used (6, 21, 36). Dry-ashing physiological specimens at low temperatures is, at best, time-consuming. Muffle space and the price of platinum would limit the use of such methods on a large scale.

Several wet-ashing procedures have been reported. The use of sulfuric and nitric acid combinations has been reported in the treatment of sewage (10, 32). Sulfuric, nitric, and perchloric acid combinations or perchloric acid alone have been used (2, 15, 30, 35). Perchloric acid rapidly oxidizes organic matter, but it has the distinct disadvantage of volatilizing chromium as chromyl chloride whether chlorides are present or not (13, 16). Brard (2), following the wet-ashing method of Kahane (16), used a perchloric acid method to digest 200- to 300-gram samples of animal tissues. The escaping gases were passed through a condenser and returned to the original flask. This method requires a great deal of attention and is too cumbersome to be used for a large number of samples. The end of the digestion is difficult to judge with some samples, and the digestion products are difficult to concentrate, without loss of chromium, to the small volumes necessary for measuring minute amounts of chromium.

A considerable amount of work has been reported on the ashing of physiological specimens for the determination of lead (18, 20, 40). A study of these methods showed that wet ashing could be accomplished in glass beakers and in a comparatively short time. After experimenting with several different methods, the authors adopted a combination wet- and dry-ashing procedure using borosilicate glass beakers.

OXIDATION OF CHROMIUM

The oxidation of chromium in physiological specimens has been accomplished by means of permanganate (21), persulfate (2, 28, 36), perchloric acid (35), hydrogen peroxide (26), sodium peroxide (10, 17), and various fusion mixtures (7, 31). For the authors' purposes, oxidation in an alkaline medium was desirable, as it would afford a convenient means of removing iron, manganese, and other substances insoluble in an alkaline medium. At first, hydrogen peroxide was used, but this was found to be undependable (12). The last traces of peroxide were difficult to decompose, and when the sample was made acid, just before the addition of the dye, the hydrogen peroxide would reduce the chromate ion (19). Oxidation with bromine in alkaline solution (12, 14, 22) worked more satisfactorily, and the excess bromine was easily removed with phenol, which reacts to form tribromophenol.

The relatively large amounts of phosphorus (as phosphate), calcium, and magnesium in the urine ash made an alkaline oxidation impractical because of the voluminous precipitate formed. A persulfate oxidation in acid solution with silver nitrate as a catalyst was used successfully. An objection to this method was that the developed color would fade rather quickly. In the meanwhile, it was found that sodium bismuthate, ordinarily used to oxidize manganese in strongly acid solution (1, 4), would, under the proper conditions, oxidize chromium (33). The authors were unable to find any report of previous work on the use of sodium bismuthate in the quantitative determination of chromium. However, after a number of preliminary studies, the proper conditions were found by which satisfactory results were obtained.

REAGENTS

Unless otherwise specified, all reagents are of analytical quality. Standard chromate solution was made by dissolving 0.2829

gram of potassium dichromate obtained from the National Bureau of Standards in double-distilled water and making up to 1 liter. One milliliter of this solution is equivalent to 100 micrograms of chromium. Solutions containing 10 and 1 microgram per milliliter are made by diluting this stock solution with double-distilled water.

Bromine–sodium hydroxide oxidizing solution, 6 ml. of saturated bromine water per 100 ml. of 1 N sodium hydroxide solution.

Phenol, 1.2% solution of redistilled phenol prepared with double-distilled water and stored in an amber bottle.

Diphenylcarbazide, 0.25% solution in 1 to 1 acetone and double-distilled water.

Sulfuric acid solution, 25% by volume in double-distilled water. Solution must be free of reducing substances.

Nitric acid, specific gravity 1.42, redistilled.

Distilled Water. Ordinary distilled water does not contain noticeable amounts of chromium. However, there is always a possibility that the water may contain free chlorine, organic matter, or other volatile substances which may react with the chromate ion. Once the chromium is oxidized, it is necessary to use fresh double-distilled water or double-distilled water that has been well kept.

Glassware. All glassware should be rinsed with a strong acid solution, preferably aqua regia, after it has been cleaned and before its final rinse with distilled water. The common sulfuric acid–chromic acid cleaning solution must be rigorously avoided. Beakers gradually become etched, but may be used repeatedly if properly cleaned. Beakers used for high chromium-containing samples should not be used for low chromium-containing samples.

APPARATUS

All measurements were made with a Beckman Model DU quartz spectrophotometer using 1-cm. cells. Wave length was set at 540 mμ and slit width at 0.04 mm.

ANALYTICAL PROCEDURE

Blood and Tissues. Put 10 to 20 grams of weighed sample (tissues should be well washed beforehand) in a 100- or 150-ml. beaker, and add 2 to 3 ml. of concentrated sulfuric acid and 10 ml. of concentrated nitric acid. Mix by rotating the beaker, cover with a watch glass, and heat cautiously on a hot plate until the tissues have gone into solution. Lung and liver tissues sometimes react violently with hot nitric acid. Blood, brain, and fatty tissues tend to foam; carefully treat these tissues with sulfuric acid until they are charred before adding the nitric acid. Gradually increase heat and let evaporate to sulfur trioxide fumes. Fume for 0.5 hour, remove from hot plate, cool slightly, add 5 ml. of concentrated nitric acid, return to hot plate, and again heat to fumes of sulfur trioxide. If the remaining organic matter is large, repeat the nitric acid treatment. Remove the beaker cover, fume to dryness, and place the beaker in a muffle furnace at 550° C. for 0.5 hour. The ash should have a reddish to white color. Should black spots, or a grayishness, be seen, remove the beaker from the muffle, place on the hot plate to cool, digest with 2 ml. of nitric acid, dry, and again return to the muffle. Repeat if necessary.

If a muffle is not available, ash the tissues completely by alternately treating with nitric acid and heating with a Fisher burner to just short of redness. Beakers of 100- and 150-ml. size do not easily break upon cooling after being heated strongly, but precautions should be taken to let the beakers cool on a warm surface, such as that of a hot plate or a warm asbestos pad.

After ashing is complete, add 1 ml. of concentrated hydrochloric acid, rotate to wet all of the ash, add 2 ml. of concentrated nitric acid, cover with a watch glass, heat, and allow vapors to wash down sides of beaker for a few minutes. Remove cover and evaporate to dryness. Wash down sides of beaker with approximately 10 ml. of water, using a very fine stream of water, and again evaporate to dryness. Remove from hot plate, and add approximately 25 ml. of water and 2 ml. of bromine–sodium hydroxide oxidizing solution. This should precipitate all the iron and make the solution definitely alkaline. If not, add 1 N sodium hydroxide solution until the sample is definitely alkaline. Cover, and boil gently for 0.5 hour with occasional stirring to ensure complete contact of the oxidizing solution. Evaporate to a volume of approximately 4 ml. and cool to room temperature. Transfer to a graduated 15-ml. centrifuge cone, carefully washing the insoluble residue adhering to the beaker three to four times with 1-ml. wash portions of double-distilled water from a wash bottle delivering a very fine stream of water. It is not necessary that all of the precipitate be washed into the centrifuge cone, but the precipitate should be well washed, and the total volume of the sam-

ple and washings must not exceed 8.5 ml. Centrifuge until firmly packed and decant into a 10-ml. volumetric flask. Small amounts of the precipitate escaping into the volumetric flask do not greatly affect the results; however, it is best to avoid this as much as possible. To the flask add 0.5 ml. of 25% sulfuric acid solution, making the solution 0.2 to 0.3 N. Shake, check to see that an excess of bromine (indicated by its color) is present, add 0.5 ml. of phenol solution, and shake to remove all free bromine. Add 0.5 ml. of diphenylcarbazide solution, make up to mark, shake, and take readings at a wave length of 540 mμ on a spectrophotometer or compare colorimetrically with previously prepared standards. If colors developed are too intense for reading, make proper dilutions.

Urine. To the entire urine sample add enough concentrated nitric acid to equal 5% of the volume of the sample. Mix thoroughly and let sit for at least 2 hours. Take an aliquot equivalent to 50 ml. of urine, add 5 ml. of concentrated nitric acid, cover with watch glass, heat cautiously until solution has cleared, boil to near dryness, and take to complete dryness with care, as the reaction is usually very rapid at this point. Cool, add 2 to 3 ml. of concentrated nitric acid, cover with watch glass, and again evaporate to dryness. Remove cover, and place beaker in muffle at 500° C. for 20 minutes. Cool and add 2 ml. of concentrated nitric acid and 2 drops of concentrated phosphoric acid. Cover, heat for a few minutes to allow acid vapors to bathe sides of beaker, remove cover, and wash sides of beaker with 10 to 15 ml. of water. Stir by rotation until ash is completely dissolved and carefully evaporate to dryness without visible boiling. At this point the sample tends to spatter, but this can be avoided by agitating beaker just as the sample comes to dryness. Let sample bake for a few minutes and then place in the muffle for 10 minutes. Cool, add 5 ml. of water from a graduate and 1 ml. of 25% sulfuric acid. Stir until completely in solution and then add approximately 50 mg. of sodium bismuthate. Mix thoroughly by rotation, cover with a watch glass, and put on a water bath for a timed 20 minutes. Stir occasionally to ensure complete contact. Remove, cool, and wash into centrifuge cone as for blood and tissues; do not let the volume exceed 8.5 to 9.0 ml. Rinse inside of cone while making volume up to 9.5 ml. Centrifuge until the bismuthate is tightly packed and carefully decant into a 10-ml. volumetric flask. Add 0.5 ml. of diphenylcarbazide solution, make up to mark, mix, and read as for tissues. Blanks using urine from unexposed persons should be run simultaneously with each batch of samples.

STANDARDIZATION

Table I gives the optical density values obtained from duplicate samples of blood and urine which were run through the procedure as given. Ten milliliters of whole blood or 50 ml. of urine were used for each sample. The blood was obtained through the cooperation of the local American Red Cross chapter, and the urine was obtained from unexposed laboratory personnel. The chromium was added by means of a microburet.

Table I. Optical Density Values Obtained from Duplicate Blood and Urine Samples

	Chromium Added, Micrograms									
	0	0.10	0.20	0.50	0.80	1.00	2.00	5.00	8.00	10.0
Blood	0.009	0.017	0.026	0.048	0.071	0.082	0.158	0.362	0.610	0.755
	0.010	0.019	0.024	0.045	0.068	0.083	0.160	0.367	0.600	0.745
Urine	0.011	...	0.025	0.045	0.066	0.085	0.155	0.360	0.605	0.720
	0.013	...	0.026	0.047	0.070	0.080	0.151	0.355	0.595	0.730

Figure 1 compares the standard curves obtained from these values, after blank corrections, with the curve obtained from pure standard dichromate solution in 10 ml. of 0.2 N sulfuric acid solution. Recovery is relatively good and there is close agreement between the blood and urine curves. The deviations from the standard solution curve are probably due to the effects of the extraneous substances in the actual samples as well as the small amounts of chromium left in the centrifuge tubes.

Confirmation with Beer's law up to a concentration of 10 micrograms of chromium per 10 ml. of final solution (or 1 p.p.m.) is also shown. The molar extinction coefficient of the colored compound, based on the molarity of a dichromate solution, is 8.32 ×

10^4. Theoretically, this should enable the spectrophotometer to detect differences of 0.002 microgram of chromium per milliliter of solution. However, detection of differences of 0.005 microgram of chromium per milliliter of solution is the practical limit of sensitivity.
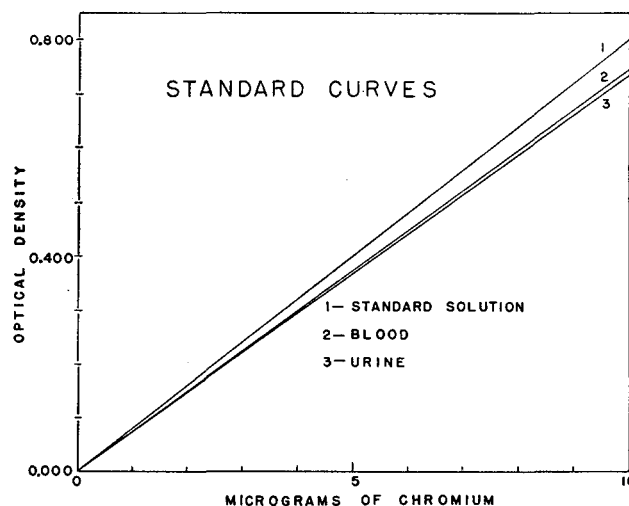

Figure 1. Comparison of Standard Solution Curve with Curves Obtained from Blood and Urine Standards

Table II shows the percentage of chromium recovered from approximately 10 grams of wet tissue taken from autopsy specimens obtained from persons known to be nonchromate workers. The specimens were furnished through the cooperation of the Department of Pathology, The Ohio State University. Three sections, as uniform as possible, were cut from each specimen, and to each section 0, 1, or 2 micrograms of chromium were added. The sections were then analyzed as above. The original chromium in the sections to which chromium was added was calculated from the analysis of the section to which no chromium was added. This assumed a uniform distribution of the original chromium throughout the autopsy specimen, but such an assumption is not necessarily valid. However, the sections were fairly uniform, and the recoveries consistently fell within 10% of the amount added.

Trivalent chromium, as the nitrate, was also run through the procedures with blood and urine samples. Inasmuch as there were no notable differences in the results, the potassium dichromate salt was used as a standard because of its higher purity.

Figure 2 compares the optical density versus the time curve of a standard dichromate solution with the curves obtained from urine and blood standards. As can be seen, the urine samples fade rather rapidly. This is due, in part, to the greater acidity of the final solution combined with the effects of small traces of sodium bismuthate which are carried over with the decantate. Attempts to eliminate this fading with sodium azide (9) were unsuccessful. However, good results can be obtained by taking readings within 5 minutes after the diphenylcarbazide has been mixed with the sample. Blood samples should be read within 20 minutes after mixing.

DISCUSSION

More than 1000 experimental samples were run in the development of these procedures. Although the recommended sample sizes give optimum results, the size of the samples actually analyzed varied from 0.1 up to 30 grams of blood or tissue and from 10 to 100 ml. of urine. Blood samples were collected with special platinum needles and evacuated-type vials. Urine samples were collected in specially prepared glass jars.

The amounts of chromium found varied from undetectable amounts up to 3 micrograms per 10 grams of blood, 450 micrograms per 10 grams of tissue, and 35 micrograms per 100 ml. of urine. The usual amounts encountered, however, were in the neighborhood of 0.5, 1, and 5 micrograms, respectively.

Among the tissues and organs analyzed were lung, kidney, liver, spleen, brain, heart, stomach, adrenal glands, lymph nodes, pancreas, thyroid, large and small intestines, muscle, fecal matter, malignant tumors, and small pieces of bone and cartilage. Tissues which contain large amounts of calcium and phosphorus, such as bone and cartilage, form a voluminous precipitate which interferes with the oxidation of the chromium. The authors were able to get some satisfactory results by extending the period of boiling with the bromine–sodium hydroxide solution; however, the method is not recommended for such tissues without further study.

By preparing a batch of samples in the afternoon of one day, letting them digest on a sand bath or a slightly warm hot plate overnight, and finishing the batch the next day, a single laboratory technician can run fifteen to twenty samples a day. Substances, such as selenium and ashing aids (38) which assist in the decomposition of organic matter, were not tried because of the possibility of interference and/or contamination.

Most difficulties can be traced to improperly or incompletely ashed samples. Chromium is not easily volatilized under the conditions outlined, and low results are usually due to incomplete oxidation of either the organic matter or the chromium rather than to a loss of chromium.
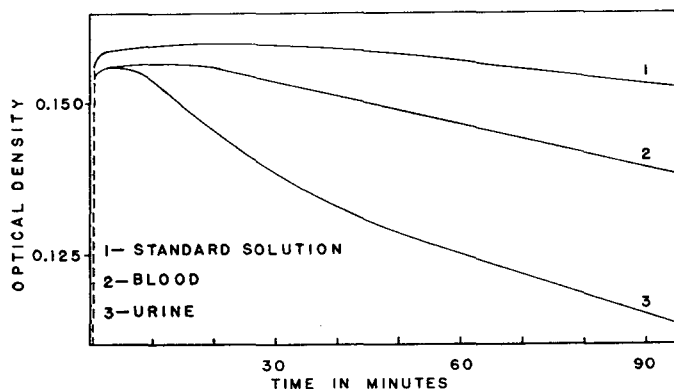

Figure 2. Comparative Fading Rates of 2 Micrograms of Chromium in 10 Ml. of Final Solution

After the samples have been ashed, the hydrochloric and nitric acid treatment completely dissolves the blood ash, the ash of most tissues, and any chromium oxides that may have been formed. The nitric acid, which is in excess, removes most of the chlorine and chlorides, while the subsequent evaporation with water removes most of the free acid. The phosphoric acid which is added to the urine samples compensates, in part, for variations in urine content and aids in making the ash soluble.

The sodium bismuthate oxidation of the urine samples should be carefully controlled. The method as given is somewhat flexible, but excessive variations in time, temperature, or acidity may cause either high results due to side reactions or incomplete oxidation of the chromium. Chlorides and fluorides must be absent, and the amount of sodium bismuthate added to each sample should be approximately uniform. This may be accomplished by means of a small scoop made of glass tubing.

Centrifuging the excess sodium bismuthate out of the sample should also be done with some care. If a centrifuge is not available, or if slightly greater accuracy is desired, the samples may be filtered directly into the 10-ml. volumetric flasks, using approved microtechniques (24, 25).

Should the chromium-diphenylcarbazide color be too intense

Table II. Chromium Recovery from Unexposed Autopsy Specimens

Tissue	Sample Weight	Cr. Added	Cr Found	Cr in Tissue	Added Cr Recovered
	Grams	γ	γ	γ	%
Lung	10.0	0	1.30	1.30	...
	11.1	1.00	2.40	1.45ᵃ	95
	10.2	2.00	3.15	1.33ᵃ	91
Liver	9.0	0	0.05	0.05	...
	9.9	1.00	1.10	0.06ᵃ	104
	7.6	2.00	1.95	0.04ᵃ	96
Spleen	9.6	0	0.15	0.15	...
	10.9	1.00	1.10	0.17ᵃ	93
	9.7	2.00	2.05	0.15ᵃ	95
Kidney	9.4	0	0.80	0.80	...
	10.1	1.00	1.90	0.86ᵃ	104
	10.7	2.00	2.90	0.91ᵃ	99

ᵃ Calculated.

for reading, the contents of the 10-ml. flask may be rinsed into a larger volumetric flask, and the reading taken after proper adjustments for acidity and diphenylcarbazide concentration. In doing this, the loss of accuracy is not very great. If greater accuracy is desired, the blood and tissue samples with their centrifugates may be returned to their original beakers, reashed, and reprocessed using larger volumetric flasks or an aliquot of the samples for color development. Urine samples should be rerun entirely using smaller samples.

A 0.25% solution of diphenylcarbazide in alcohol may be used in place of the recommended acetone solution. In either case the solution becomes brown upon standing. The brown color seems to have no noticeable effect, and with good grades of acetone the coloring effect is slow. Ege and Silverman (8) have recently reported the use of phthalic anhydride as a stabilizer for the diphenylcarbazide solution; nevertheless, the authors made their solution fresh daily.

INTERFERENCES

The diphenylcarbazide method is almost specific for chromium. Ferric iron, which gives a yellow color with diphenylcarbazide, is eliminated in the method for blood and tissues. In the method for urine, what iron may be present will give little if any interference because of its low sensitivity. Manganese, as permanganate, reacts with low sensitivity to give a faintly yellow color. Very large amounts of permanganate should be reduced with a few granules of sodium azide (9) before the dye is added. The interference of vanadium is a more serious possibility, because it gives a stronger yellow color than does iron. If the ratio of vanadium to chromium does not exceed 10 to 1, nearly correct results for chromium can be obtained by allowing the solution to stand for about 10 minutes after the addition of the reagent, as the vanadium-diphenylcarbazide color fades fairly rapidly. In amounts greater than this, the vanadium may be removed by treating with 8-hydroxyquinoline and extracting with chloroform (29).

ACKNOWLEDGMENT

LITERATURE CITED

(1) Blum, W., J. Am. Chem. Soc., 34, 1379 (1912).
(2) Brard, Daniel, "Actualités Scientifiques et Industrielles," No. 228, Paris, Herman et Cie., 1935.
(3) Cazeneuve, P., Bull. soc. chim. Paris, [3] 23, 701 (1900); [3] 25, 761 (1901).

(4) Cunningham, T. R., and Coltman, R. W., *Ind. Eng. Chem.*, **16**, 58 (1924).
(5) Davis, H. C., and Bacon, A., *J. Soc. Chem. Ind. (London)*, **67**, 316 (1948).
(6) Dingwall, A., and Beans, H. T., *Am. J. Cancer*, **16**, 1499 (1932).
(7) Dingwall, A., Crosen, R. G., and Beans, H. T., *Ibid.*, **21**, 606 (1934).
(8) Ege, J. F., Jr., and Silverman, L., ANAL. CHEM., **19**, 693 (1947).
(9) Feigl, F., "Qualitative Analysis by Spot Tests," 3rd ed., pp. 128–32, New York, Elsevier Publishing Co., 1946.
(10) Graham, D. W., *J. Am. Water Works Assoc.*, **35**, 159 (1943).
(11) Gutzeit, G., *Helv. Chim. Acta*, **12**, 713 (1929).
(12) Heller, K., and Krumholz, P., *Mikrochimie*, **7**, 220 (1929).
(13) Hoffman, J. I., and Lundell, G. E. F., *J. Research Natl. Bur. Standards*, **22**, 465 (1939).
(14) Järvinen, K. K., *Z. anal. Chem.*, **75**, 1 (1928).
(15) Kahane, Ernest, "Actualités Scientifiques et Industrielles," "L'Action de l'Acide Perchlorique sur les Matières Organiques," No. 167, "Généralités," No. 168, "Applications," Paris, Hermann et Cie., 1934.
(16) Kahane, E., and Brard, D., *Bull. soc. chim. biol.*, **16**, 710 (1934).
(17) Kamerman, P. A. E., *S. African J. Sci.*, **41**, 165 (1945).
(18) Letonoff, T. V., and Reinhold, J. G., IND. ENG. CHEM., ANAL. ED., **12**, 280 (1940).
(19) McAlpine, R. K., and Soule, B. A., "Qualitative Chemical Analysis," p. 315, New York, D. Van Nostrand Co., 1933.
(20) Marriott, W. R. V., *Am. J. Clin. Path.*, **12**, 488 (1942).
(21) Maunsell, P. W., *New Zealand J. Sci. Tech.*, **26B**, 94 (1944).
(22) Miller, C. C., *J. Chem. Soc.*, **1943**, 72.
(23) Moulin, A., *Bull. soc. chim. Paris*, [3] **31**, 295 (1904).

(24) Niederl, J. B., and Niederl, V., "Micromethods of Quantitative Organic Analysis," p. 153, New York, John Wiley & Sons, 1942.
(25) Pregl, F., "Quantitative Organic Microanalysis," p. 89, London, J. & A. Churchill, 1945.
(26) Rossi, L., Marenzi, A. D., and Lobo, R., *Anales farm. bioquim. (Buenos Aires)*, **13**, 1 (1942).
(27) Rowland, G. P., IND. ENG. CHEM., ANAL. ED., **11**, 442 (1939).
(28) Saint Rat, L. de, *Compt. rend.*, **227**, 150 (1948).
(29) Sandell, E. B., "Colorimetric Determination of Traces of Metals," pp. 191–9, New York, Interscience Publishers, 1944.
(30) Smith, G. F., "Mixed Perchloric, Sulphuric, and Phosphoric Acids, and Their Applications in Analysis," Columbus, Ohio, G. Frederick Smith Chemical Co., 1942.
(31) Snoddy, A. O., *J. Oil & Fat Inds.*, **2**, 20 (1925).
(32) Stones, T., *Inst. Sewage Purif., J. and Proc.*, **1944**, 241.
(33) Treadwell, F. P., and Hall, W. T., "Analytical Chemistry," Vol. I, p. 201, New York, John Wiley & Sons, 1937.
(34) Urone *et al.*, ANAL. CHEM., **22**, 472 (1950).
(35) Verbeck, B. J., *Chemist-Analyst*, **29**, 81 (1940).
(36) Walt, C. F. J. van der, and Merwe, A. J. van der, *Analyst*, **63**, 809 (1938).
(37) Welcher, F. J., "Organic Analytical Reagents," Vol. III, pp. 430–47, New York, D. Van Nostrand Co., 1947.
(38) *Ibid.*, p. 495.
(39) Wenger, P., Duckert, R., and Rusconi, Y., *Helv. Chim. Acta.*, **27**, 1839 (1944).
(40) Winn, G. S., *J. Ind. Hyg. Toxicol.*, **27**, 178 (1945).

Partition Chromatography of Synthetic Anthocyanidin Mixtures

EARL C. SPAETH AND DAVID H. ROSENBLATT

University of Connecticut, Storrs, Conn.

A method has been developed for the quantitative separation and analysis of small amounts of mixtures of the synthetic anthocyanidins, malvidin, petunidin, and delphinidin. Separation is accomplished by partition chromatography on columns of silicic acid with 10% phosphoric acid as the immobile aqueous phase and a mixture of phenol and toluene as the nonaqueous phase. R values are reported for the anthocyanidins. The anthocyanidin solutions after separation obey Beer's law. The concentrations of these solutions are determined spectrophotometrically.

DURING the course of an investigation into the nature of the colored substances of wine and grapes, it was necessary to consider existing procedures for the separation and quantitative analysis of small amounts of various mixtures of anthocyanins and anthocyanidins. It has been shown by previous workers (*1, 7, 10, 15, 17, 25*) that the skins of some colored grapes contain anthocyanins which are mono- or diglucosides of the anthocyanidins, malvidin, petunidin, and delphinidin.
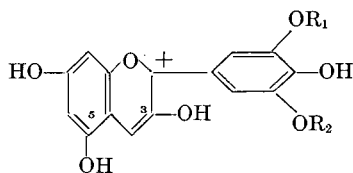
represents malvidin when $R_1 = R_2 = CH_3$, petunidin when $R_1 = CH_3$ and $R_2 = H$, and delphinidin when $R_1 = R_2 = H$. The naturally occurring glucosides of these anthocyanidins usually

have glucose residues attached to the 3 or to the 3 and 5 positions. These substances are usually isolated as chlorides or picrates. In grapes or wine, the anion may be some organic ion such as tartrate. During the process of the transformation of grapes into aged wine, part of this anthocyanin mixture is probably hydrolyzed to anthocyanidins and a gradual demethylation probably occurs, so that the concentration of delphinidin or its glucosides is increased at the expense of malvidin and petunidin or their glucosides (*10*).

Mixtures of anthocyanins have been separated by Karrer and his co-workers. A mixture of the picrates of the monoglucosides of malvidin, petunidin, and delphinidin obtained from the fruit of the bilberry was partly resolved by 31 recrystallizations (*16*). A similar mixture, "althaein," obtained from the flowers of the black mallow, was partly resolved by adsorption chromatography on a column of calcium sulfate (*13, 14*). Adsorption chromatography of red wines has also been reported (*11, 19*), and although separate colored bands have been obtained on columns of adsorbants, no attempts at the identification of the pigments in these bands were mentioned. On the basis of these publica-

tions, neither fractional crystallization nor adsorption chromatography seemed suitable for the quantitative separation of small amounts of anthocyanin or anthocyanidin mixtures. No other methods for such separations had been reported at the time the present work was started.

It seemed reasonable that a method for the resolution of a mixture of anthocyanins and anthocyanidins, such as might be found in a sample of red wine, could be based on differences in partition coefficients of the pigments in various pairs of immiscible solvents. Such differences have already been reported. Willstätter (26), for example, has based a procedure for the isolation of a relatively pure specimen of oenin chloride, a monoglucoside of malvidin, on the observation that when a dilute aqueous hydrochloric acid solution of a mixture of anthocyanins and anthocyanidins is shaken with amyl alcohol, all of the anthocyanidins and part of the monoglycosides will pass to the amyl alcohol layer, while little, if any, of the diglycosides will leave the aqueous acid layer. Robinson and Robinson (21) have found solvent systems which will distinguish between various anthocyanidins. For example, when 1% hydrochloric acid solutions of anthocyanidins are shaken with a mixture of 1 volume of ethyl isoamyl ether and 4 volumes of anisole containing 5% picric acid, pelargonidin, peonidin, and malvidin are completely extracted, cyanidin and petunidin are partly extracted, and delphinidin is not extracted from the acid aqueous layer.

With these experiments in mind, it seemed worth while to consider using either the technique of partition chromatography developed by Martin and Synge (18) or the countercurrent distribution technique of Craig (8) to effect the desired separations. For this present work, partition chromatography was chosen. After the present work was under way, Bate-Smith (2, 3) and Forsyth (9) reported filter-paper chromatography of natural and artificial mixtures of anthocyanins and anthocyanidins. The present authors have also made a preliminary report (23) which indicates that separation of malvidin and petunidin can be effected by partition chromatography.

Because the pigments of wine and grapes were eventually to be examined, it was decided to develop first a procedure for the separation of mixtures of malvidin, petunidin, and delphinidin. Anthocyanidins rather than anthocyanins were chosen for the following reasons: Pure samples of anthocyanidins would be easier to obtain by synthesis. It has long been known that anthocyanins can be hydrolyzed quantitatively to anthocyanidins and the sugar. It has been shown that anthocyanidins and anthocyanins can be separated easily as groups by a series of distributions between amyl alcohol and dilute hydrochloric acid (26). A method for the separation and quantitative analysis of mixtures of malvidin, petunidin, and delphinidin therefore could give considerable information concerning the nature of mixtures of related anthocyanins after separation from the anthocyanidins and hydrolysis. On the other hand, ability to separate a mixture of monoglucosides or a mixture of diglucosides would be of little assistance in obtaining information concerning the nature of an accompanying related anthocyanidin mixture.

The procedures as outlined in the present work show that separation and quantitative recovery of the components of microgram quantities of anthocyanidin mixtures containing malvidin, petunidin, and delphinidin can be accomplished by partition chromatography, using silicic acid as a column support, 10% phosphoric acid as the nonmobile phase, and a mixture of phenol and toluene as the mobile phase.

GENERAL PROCEDURE

A freshly dried sample of silicic acid of suitable particle size is ground in a mortar with enough of the aqueous phase (10% phosphoric acid saturated with nonaqueous phase) so that the ratio of milliliters of aqueous phase to grams of silicic acid is 0.55. The resulting powder is transferred to a separatory funnel containing

about 7 times as much of the nonaqueous phase (a solution of w grams of phenol and w/2 ml. of toluene saturated with aqueous phase) as the aqueous phase taken. After shaking, the resulting slurry is allowed to settle briefly with occasional rocking to eliminate air bubbles and is then transferred with the aid of a small amount of additional nonaqueous phase to a chromatography tube. Air pressure of about 30 cm. of mercury is applied to the top of the tube and the tube is tapped occasionally to assure even settling. When the nonaqueous solvent just disappears below the surface of the silicic acid, the air pressure is released.
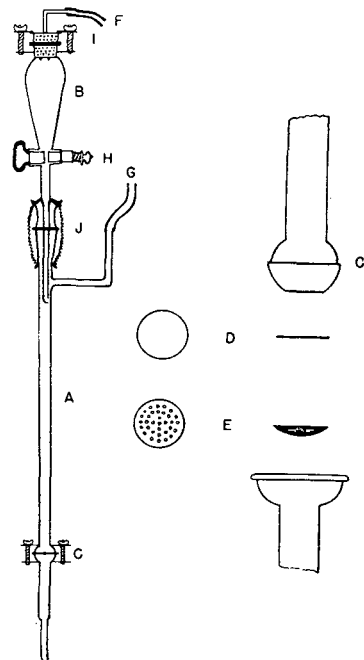

Figure 1. Diagram of Apparatus

A mixture of synthetic malvidin, petunidin, and delphinidin chlorides is dissolved in a little methanol containing 0.25% orthophosphoric acid and the solution is diluted to a known volume with nonaqueous phase. An aliquot of this solution is placed carefully on the column so that the surface of the silicic acid is not disturbed. Pressure is reapplied and when the pigment-containing solution has sunk completely into the surface of the column, additional nonaqueous phase is added to develop the pigment bands. As a single red band at the top of the column moves down the column, it separates into three bands which continue to move at different rates. The R values (18) of these bands are determined. Development with nonaqueous phase is continued until each band is eluted completely from the column. The three separate colored portions of the eluate are collected in volumetric flasks and diluted to known volumes. The concentration of the anthocyanidin in each solution is measured spectrophotometrically.

DEVELOPMENT OF METHOD

Synthesis of Anthocyanidins. MALVIDIN CHLORIDE. This anthocyanidin was synthesized and isolated as the chloride according to the directions of Hayashi (12). The malvidin chloride was dried over solid potassium hydroxide and concentrated sulfuric acid at 25° C. in vacuo. Calculated for $C_{17}H_{15}O_7Cl.H_2O$, C 53.06, H 4.45, Cl 9.22, OCH$_3$ 16.1%. Found, C 52.95, 52.76, H 4.37, 4.40, Cl 10.42, 10.59, OCH$_3$ 17.36, 17.42. Reported analytical carbon and hydrogen values correspond to a monohydrate (12). The shape of the crystals of malvidin chloride was the same as that pictured by Hayashi (12). The intermediate ω,4-diacetoxy-3,5-dimethoxyacetophenone had a melting point of 123°, reported 123° (12). Calculated for $C_{12}H_{10}O_5(OCH_3)_2$, OCH$_3$ 20.95%. Found, OCH$_3$ 21.14%.

PETUNIDIN CHLORIDE. This anthocyanidin was synthesized and isolated as the chloride according to the directions of Robinson and co-workers (5) with two following modifications. The

acid chloride of 3-methoxy-4,5-diphenylmethylenedioxybenzoic acid was prepared with thionyl chloride in 74% yield. The melting point was 109°. This sample gave a negative qualitative sulfur test and no depression in melting point when mixed with a sample prepared with phosphorus pentachloride as reported (5). Reported melting point 109° C. (5). The condensation between benzoylphloroglucinaldehyde and ω-acetoxy-3-methoxy-4,5-diphenylmethylenedioxyacetophenone to form benzoylpetunidin (6) was greatly facilitated by addition of small amounts of absolute ethyl alcohol to the reaction mixture (15 ml. of ethyl alcohol for 300 ml. of ethyl acetate). The petunidin chloride was dried over solid potassium hydroxide and concentrated sulfuric acid at 25° in vacuo. Calculated for $C_{16}H_{13}O_7Cl.1.5H_2O$, C 50.60, H 4.25, Cl 9.34, OCH_3 8.17%. Found, C 50.17, 50.47, H 4.27, 4.19, Cl 8.60, 8.84, OCH_3 5.82, 5.94%. A second sample of petunidin chloride prepared as just described gave the following analyses: C 50.97, 50.56, H 4.30, 4.21, Cl 9.19, 9.30, OCH_3 9.04, 8.70%. The intermediate ω-acetoxy-3-methoxy-4,5-diphenylmethylenedioxyacetophenone had a melting point of 125–127°, reported 126–127° (5). Calculated for $C_{23}H_{17}O_5(OCH_3)$, OCH_3, 7.67%. Found, OCH_3 7.89%.

DELPHINIDIN CHLORIDE. This anthocyanidin was synthesized according to the directions of Robinson and co-workers (5). The crude delphinidin chloride was not purified as reported but was washed with ether and 20% hydrochloric acid, and was recrystallized according to Hayashi (12), except that methanol was substituted for the ethyl alcohol used. The delphinidin chloride was dried over solid potassium hydroxide and concentrated sulfuric acid at 25° in vacuo. Calculated for $C_{15}H_{11}O_7Cl.2H_2O$, C 48.07, H 4.04, Cl 9.46%. Found, C 47.67, 47.81, H 4.48, 4.44, Cl 9.51, 9.86%.

These three anthocyanidin chlorides gave the same colors and behavior with a series of buffered solutions and with other test reagents as those reported (5, 20, 21) within the limits imposed by the lack of authentic specimens for comparison. No reasonable explanation seems to be available for the discrepancies between calculated and found values for the methoxyl analyses in these compounds.

Two-Phase Solvent System. To aid in choosing a suitable two-phase solvent system, the following criteria were used: The anthocyanidins should be appreciably soluble in the nonaqueous mobile phase. The aqueous phase should contain a strong acid in sufficient concentration to prevent isomerization of the flavylium salt to the colorless modification. The organic solvents should not be so volatile that changes in composition of the nonaqueous phase would occur rapidly during the manipulations. The anthocyanidins should differ with respect to their partition coefficients in the two-phase system.

Many two-phase systems made up of various aqueous acids, organic solvents, and organic solvent pairs were tested. Those systems which dissolved appreciable amounts of the anthocyanidins and in which the anthocyanidins had reasonable partition coefficients were tested further by partition chromatographic experiments using silicic acid columns. Although several two-phase systems seemed to be suitable, it was found that 10% phosphoric acid and a mixture of w grams of phenol and $w/2$ ml. of toluene satisfy all the above criteria and permit pigments to move down a column at a satisfactory rate without excessive increase in width. Although a decrease in the proportion of toluene in this system permits preparation of more concentrated nonaqueous phase solutions, the partition coefficients are also changed, so that the nonaqueous phase is favored. The latter change is undesirable because pigment bands on the column then become more diffuse during development, and the relative rates of movement of the bands become more nearly equal. In the authors' hands, better separations were obtained with 10% phosphoric acid as the aqueous phase than with 1 or 10% hydrochloric or sulfuric acids. All the fourteen other aqueous mineral or organic acids tested were unsatisfactory for various reasons.

A mixture of w grams of freshly distilled phenol (Paragon practical grade), $w/2$ ml. of toluene (Baker and Adamson, U.S.P. grade), and w ml. of 10% phosphoric acid (by dilution of Baker and Adamson reagent grade, 85%) is shaken several minutes in a separatory funnel. The two phases are separated and the non-

aqueous phase is filtered twice through filter paper. The solutions are preserved and are stable in brown bottles. It is recommended that the equilibration be made a few degrees below the temperature at which the solvent system will be used. Otherwise, a small temperature decrease will cause separation of water droplets from the nonaqueous phase. When 50 ml. of nonaqueous phase prepared as described above are warmed to a temperature 3° above that of equilibration, it will dissolve less than one drop of water.

Although no accurate determinations of the solubilities of the three anthocyanidin chlorides in the nonaqueous phase were made, experiments show that concentrations of approximately 150, 300, and 600 mg. per liter of solution can be obtained for delphinidin, malvidin, and petunidin, respectively. For chromatographic separations, however, upper limits of 60 mg. per liter for delphinidin, and 150 mg. per liter for malvidin and petunidin are recommended. Solubility of these anthocyanidins in the aqueous phase is a more complex matter. It was found that 1 to 3 mg. of any of the three anthocyanidin chlorides will dissolve rapidly at room temperature in 1 ml. of aqueous phase. After these aqueous solutions stand at room temperature for several hours, however, the petunidin and delphinidin solutions deposit precipitates which are much less soluble in the aqueous phase. A solution of malvidin in aqueous phase does not deposit such a precipitate, even after 2 weeks at room temperature. It is possible that these precipitates are anthocyanidin phosphates. It is not possible to state at this time whether such precipitates are formed during a chromatographic separation.
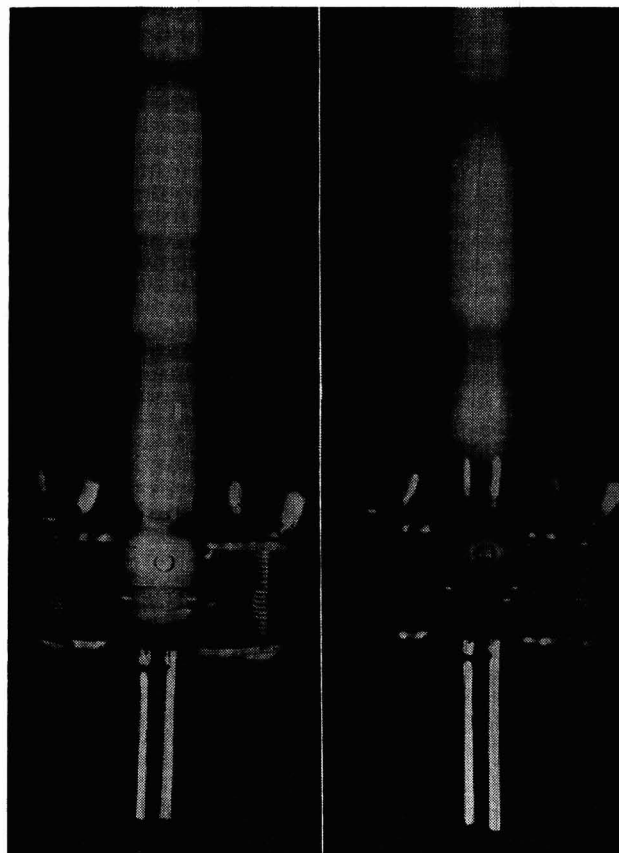

Figure 2. Two Views of Same Column

Silicic Acid. The inert solid used to hold the immobile phase is silicic acid, modified according to the procedure of Bradfield and Penney (4) and dried as described below. Columns prepared from two samples of silicic acid purchased at 2-year intervals and modified as described gave the same analytical results

and R values within the experimental errors of the determinations.

A 200-gram sample of silicic acid (Eimer and Amend precipitated metasilicic acid; reported iron content 0.003%; reported other heavy metal content 0.000%) is stirred vigorously with 2600 ml. of distilled water and is allowed to settle for 5 minutes. After decantation, the precipitate is discarded and the remaining suspension is allowed to settle for an additional 30 minutes. The resulting precipitate (40 grams) is collected and dried at 110° C. for 8 to 15 hours. The silicic acid is redried at 110° for at least 8 hours before each use.

Apparatus. Figure 1 is a diagram of the apparatus used for the chromatographic operation.

The tube, A, 38 cm. $\times$ 8.7 mm. in inside diameter, is connected at the top to a 60-ml. addition funnel, B, with a 14/35 standard-taper joint. The bottom of A is connected by means of an 18/9 standard hemispherical joint, C, to a narrow outlet tip. Between the male and female sections of joint C, shown enlarged in Figure 1, are two disks. D is a piece of filter paper, 4 mm. larger in diameter than the lower opening in the male part of the joint. E is a perforated piece of silver foil of the same diameter as D. When these disks are clamped tightly between the parts of joint C, silicic acid is retained completely in the tube and no leakage of liquid between the surfaces of the joint is observed. Controlled air pressure of about 30 cm. of mercury is introduced to the column and addition funnel at points F and G. Clamps H and I and springs J keep the apparatus together when pressure is applied.

Chromatographic Separations. The columns used in most of the chromatographic separations were prepared as described in the General Procedure from approximately 1.4 ml. of aqueous phase and approximately 2.6 grams of silicic acid (accurate ratio of 0.55). These amounts give a 10-cm. column. Continued flow of fresh nonaqueous phase through a column gradually causes the upper part of the column to become somewhat translucent—for example, flow of 50 ml. of nonaqueous phase produced a 2-mm. layer of translucence at the top of the column. It was suggested by a referee that this phenomenon is due to the removal of water from the silicic acid. That this suggestion may be correct is shown by the following experiment. A modified nonaqueous phase containing less water than the usual nonaqueous phase was prepared from 48.5 ml. of nonaqueous phase, 1 gram of phenol, and 0.5 ml. of toluene. Flow of only 15 ml. of this modified solution produced a 2-mm. layer of translucence in the usual silicic acid column. Apparently the procedures described do not give a nonaqueous phase–aqueous phase–silicic acid system which is at equilibrium. The gradual appearance of translucence does not seem to interfere with a chromatographic separation, but it may prevent the use of a single column for several successive separations.

An accurately measured 0.3-ml. portion of solution containing 30 to 60 micrograms of total anthocyanidin mixture is usually applied to a 10-cm. column. In practice, mixtures are prepared by weighing 1 to 5 mg. of each synthetic anthocyanidin chloride so that the total pigment weight is 5 to 9 mg. Complete solution in the nonaqueous phase is facilitated and assured by preliminary solution of the dry solid mixture in 1 ml. of methanol which contains 0.25% orthophosphoric acid. The resulting solution is then diluted to 50 ml. in a volumetric flask with nonaqueous phase.

The initial band width is 5 to 6 mm. Development of the initial band with additional nonaqueous phase causes complete separation of the mixed pigment band into three bands before the "front" of the most rapidly moving band reaches the base of the 10-cm. column. Figure 2 contains two photographs of the same column at two stages in a chromatographic separation of malvidin, petunidin, and delphinidin. The most rapidly moving band is due to malvidin. The band which is moving most slowly is due to delphinidin. It is interesting that the front of each separate band is diffuse, whereas the rear or uppermost edge of each band is relatively sharp. This phenomenon was always

observed when using the silicic acid and solvent system as described. It was observed that complete chromatographic separation including elution of approximately equal amounts of malvidin and petunidin as described requires almost the entire length of a 10-cm. column. Delphinidin can be separated completely from malvidin or petunidin or a mixture of both with a 3-cm. column of silicic acid. From Figure 2, it can be seen that band widths increase as the bands move down the column. It was found that the delphinidin band increases in width to a greater extent than either of the other pigment bands during movement through a 10-cm. column.

The relative rates of band movement were measured by determination of the R values of each band. R has been defined as follows (18):

$$R = \frac{\text{movement of position of maximum concentration of solute}}{\substack{\text{simultaneous movement of surface of developing fluid in}\\ \text{empty part of tube above chromatogram column}}}$$

The value of R for a particular band is determined by measuring first the liquid level above the column and the estimated position of maximum concentration of the initial band. After a band moves at least one third of the column length and is completely separated from the other bands, the liquid level above the column and the estimated position of maximum concentration of pigment in the band are again measured.

Addition of nonaqueous phase is usually continued until all bands are eluted completely from the column. The portions of the eluate are collected in volumetric flasks of suitable size.

Table I. Concentration–Optical Density Data for Anthocyanidin Solutions

Anthocyanidin	Concentration, Mg./L.	Optical Density	Concn., Mg./L. / Optical Density
Malvidin chloride	16.03	1.48	10.8
	11.08	1.03	10.8
	6.61	0.612	10.8
	3.32	0.308	10.8
Petunidin chloride	16.38	1.53	10.7
	12.68	1.19	10.7
	9.80	0.914	10.7
	6.76	0.630	10.7
Delphinidin chloride	17.93	1.63	11.0
	11.02	1.00	11.0
	6.68	0.600	11.1
	3.37	0.305	11.0

Spectrophotometric Measurements. A Beckman Model DU spectrophotometer with 1-cm. Corex cells was used for all measurements. Solutions of malvidin, petunidin, and delphinidin chlorides in nonaqueous phase at concentrations of 15.6, 14.0, and 10.2 mg. per liter, respectively, were prepared. It was found that these three solutions had absorption maxima at 551, 549, and 547 mμ, respectively.

The concentrations of unknown nonaqueous phase solutions obtained from chromatographic separations are estimated by determination of their optical densities. In order to simplify the measurements, all solutions are examined at the intermediate wave length, 550 mμ. To estimate the concentration of anthocyanidin (as chloride) from the optical density of a solution, determinations were made of the concentration (milligrams per liter) of each pigment which would give an optical density of 1.00. This value was calculated for each pigment from optical densities of solutions at several concentrations. Table I shows the data obtained. It is evident that within the range of concentration examined, the pigment solutions obey Beer's law.

In order to calculate the concentration (milligrams per liter) of anthocyanidin as the chloride in a solution of a mixture of pigments, a chromatographic separation is carried out and the following equation is used:

Table II. Stability of Malvidin Chloride Solution to Light

(5.8 mg./liter in nonaqueous phase)

Solution	Optical Density
Original at start	0.535
In ordinary borosilicate glass, exposed to light	0.428
In low-actinic, exposed to light	0.530
In ordinary borosilicate glass, kept in dark	0.532
In low-actinic, kept in dark	0.531

$$C = \frac{DkV_1}{V_2}$$

In order to estimate the recovery of each anthocyanidin as the chloride after a chromatographic separation, the weight (milligrams) of each anthocyanidin chloride taken is found by means of the following equation:

$$W = \frac{DkV_1V_3}{V_2}$$

In these equations, the symbols have the following meanings:

C = concentration of anthocyanidin chloride, mg. per liter
D = optical density
V_1 = volume (liters) of eluate collected from a column after necessary dilution to a known volume
V_2 = volume (liters) of original solution applied to a column
V_3 = total volume (liters) of original solution
W = weight (mg.) of anthocyanidin chloride originally taken
k = a constant typical of each anthocyanidin, being the concentration (mg. per liter) of nonaqueous phase solution required to give an optical density of 1.00

The values of k for malvidin, petunidin, and delphinidin are 10.8, 10.7, and 11.0 mg. per liter, respectively.

Stability of Anthocyanidin Solutions. The colored solutions of anthocyanidins in nonaqueous phase faded appreciably on continued exposure to light. In order to test this phenomenon quantitatively and to examine the possibility of minimizing the effect of light by using glassware of Pyrex Lifetime Red low-actinic glass, the following experiment was carried out:

In each of four 100-ml. volumetric flasks, two of ordinary borosilicate glass and two of Pyrex Lifetime Red low-actinic glass, was placed a 10-ml. portion of a solution of malvidin chloride in nonaqueous phase (5.8 mg. per liter). One low-actinic glass flask and one ordinary glass flask were placed in a dark cabinet, while the other pair of flasks was allowed to stand on a desk top exposed to whatever light entered the room through two large windows with eastern exposures. Little direct sunlight fell on the flasks. After 27 hours, the optical densities of samples from each flask were determined. The results are recorded in Table II.

On the basis of this experiment, the authors used low-actinic glass flasks whenever possible and carried out the chromatographic separations in dimly lit rooms. Similar solutions of delphinidin chloride in nonaqueous phase appeared to be even more sensitive to light. Other factors may influence the stability of delphinidin solutions.

RESULTS

Table III gives the data concerning the analysis of five different mixtures of malvidin, petunidin, and delphinidin chlorides. The analyses are reported in terms of the total amounts of pigments weighed and the total amounts as calculated from the analysis data. The total amount weighed was not separated. The actual amount of mixture separated (0.306 ml. of the 50 ml. of solution) is merely a small fraction of the total weight of mixture The average error in the fifteen results involved in the separations and spectrophotometric analyses of these five mixtures is 2.1%. The largest error observed is 6.3%. It was further found that the total combined weight of anthocyanidins in a nonaqueous

phase solution of a mixture can be estimated spectrophotometrically—for example, the fourth and fifth mixtures in Table III contained weighed totals of 6.35 and 6.67 mg., respectively, in 50 ml. of solution. The optical densities of these solutions at 550 mμ indicated that 6.40 and 6.65 mg., respectively, were present in the two solutions.

The R values observed during the chromatographic separations of these five mixtures are also recorded in Table III. On the basis of the five determinations of each R value, the average values are: malvidin 1.05, petunidin 0.66, and delphinidin 0.23. The maximum deviations from each of these averages are 0.11, 0.09, and 0.05, respectively.

The separations described in Table III were made with anthocyanidin chlorides whose combustion analyses are given in this paper. Some earlier work was done with anthocyanidin chlorides for which slightly less satisfactory combustion analyses were obtained. With these pigments, R values of 0.97 ± 0.09, 0.66 ± 0.10, and 0.24 ± 0.05 were obtained with malvidin, petunidin, and delphinidin, respectively. Each of these R values represents the average of 12 to 16 determinations, some with solutions of single pigments and some with solutions of pigment mixtures. R values obtained with single pigments did not differ significantly from those obtained during separation of mixtures. Concentrations similar to those mentioned in Table III were used for these determinations.

PARTITION COEFFICIENTS

Partition coefficients of the anthocyanidins in the two-phase system may be calculated from chromatographic separation data by means of the following equation developed by Martin and Synge (18). The symbols have the meaning assigned by these authors (18).

$$\alpha = \frac{A}{RA_S} - \frac{A_L}{A_S}$$

Table III. Chromatographic Separation and Quantitative Analysis of Anthocyanidin Mixtures

M = malvidin chloride
P = petunidin chloride
D = delphinidin chloride
Weighed amounts in 50 ml. solution; 0.306-ml. portion taken for separation and analysis

Composition of Mixture, Mg.		Absolute Error, Mg.	% Error	R Obtained during Separation
By actual weight	By analysis			
M 2.81	M 2.90	0.09	3.1	1.07
P 2.52	P 2.69	0.17	6.3	0.67
D 1.63	D 1.62	0.01	0.6	0.28
M 1.22	M 1.30	0.08	6.2	0.94
P 4.81	P 4.82	0.01	0.2	0.62
D 2.12	D 2.06	0.06	2.9	0.21
M 3.61	M 3.59	0.02	0.6	1.08
P 1.70	P 1.70	0.00	0.0	0.57
D 2.03	D 2.03	0.00	0.0	0.21
M 2.51	M 2.61	0.10	3.8	1.07
P 1.12	P 1.16	0.04	3.4	0.70
D 2.72	D 2.67	0.05	1.9	0.22
M 1.54	M 1.57	0.03	1.9	1.09
P 2.10	P 2.14	0.04	1.9	0.72
D 3.03	D 3.07	0.04	1.3	0.22

The following data concerning a typical chromatographic column used during the present work are pertinent to the use of this equation: weight of silicic acid 2.75 grams, aqueous phase used 1.52 ml., height of column 10.5 cm., 5 ml. of water fill the empty tube to a height of 8.3 cm., density of silicic acid assumed to be 2.3 grams per cc. (18). From these data and from average R values of 1.05, 0.66, and 0.23 for malvidin, petunidin, and delphinidin, respectively, partition coefficients of 1.6, 3.9, and 15.7, respectively, are calculated.

Partition coefficients were also measured directly. The concentration of a nonaqueous phase solution of each anthocyanidin was measured spectrophotometrically before and after extraction in a separatory funnel with an equal volume of aqueous phase. The amount extracted by and present in the aqueous phase can be calculated from the difference between these two concentrations. Equilibration was accomplished by continual inversion of the separatory funnel for 5 minutes. Table IV lists the determinations made. Here, α has the meaning given by Martin and Synge (18), ratio of concentration of solute in aqueous phase to concentration of solute in nonaqueous phase. No regular change of partition coefficient with concentration was observed.

DISCUSSION

It seems worth while to discuss further the shape of the pigment bands observed during chromatographic separations of anthocyanidin mixtures and the discrepancies between the values of the partition coefficients of the three anthocyanidins obtained directly and those calculated from the R values of the pigment bands.

Table IV. Direct Measurement of Partition Coefficient

(C = concentration in nonaqueous phase after extraction with equal volume of aqueous phase)

Anthocyanidin	α	C, Mg./L. (Range)	No. of Detns.
Malvidin	0.10 ± 0.06	11–21	7
Petunidin	0.74 ± 0.07	2–11	4
Delphinidin	7.1 ± 0.1	1–3	3

It has been observed (22) that the partition coefficients of certain anthocyanins in the system, 0.5% hydrochloric acid–isoamyl alcohol, are not constant but increase with an increase in concentration of anthocyanin. (In this discussion, partition coefficient is the ratio of concentration in aqueous phase to concentration in nonaqueous phase.) This phenomenon has been attributed to the tendency of anthocyanins to associate in water solutions (22). This tendency would increase with an increase in concentration. If the chromatographic separations reported in the present paper are due only to simple distributions between aqueous and nonaqueous phases and if the anthocyanidins resemble anthocyanins with respect to their tendency to associate in water solution, the diffuse fronts and the sharp rear or upper edges of the bands observed during a chromatographic separation (see Figure 2) can be explained. As a pigment band diffuses during movement down a column, the portions of the band that become more dilute with respect to pigment concentration will move more rapidly as a result of the change in partition coefficient. The more concentrated parts of the band will move more slowly and the result will be a band with a sharp upper edge and a diffuse front. The assumed relationship between concentration and partition coefficient of anthocyanidins in the system, phenol-toluene–10% phosphoric acid, was not verified during the course of the present work.

Organic carboxylic acids exhibit a different behavior when separated by partition chromatography (24). A decrease in concentration of organic acid increases the partition coefficient in many two-phase systems and wide bands with sharp fronts and diffuse "tails" are observed. It has been shown further (24) that addition of a suitable nonextractable buffer mixture to the nonmobile aqueous phase will decrease the ionic dissociation of organic acids in the nonmobile aqueous phase. The change in partition coefficient with change of concentration therefore decreases and the acid bands stay relatively narrow and retain sharp edges.

It is interesting to speculate on the possibility of obtaining

sharper, narrower bands during chromatographic separations of anthocyanidins. By adding a nonextractable substance to the nonmobile phase which would decrease the amount of possible association of the anthocyanidins, the changes of partition coefficient with concentration should be reduced, and sharp, narrow bands should be obtained during chromatography. Robinson and Robinson (22) have reported work with the anthocyanin, oenin chloride, which shows that addition of papaverine hydrochloride to the system, 0.5% hydrochloric acid–isoamyl alcohol, will decrease the variations of partition coefficient with concentration of this anthocyanin.

The considerable differences observed between partition coefficients of the anthocyanidins determined directly and those calculated from R values of pigment bands seem to indicate that the chromatographic separations described in the present paper are accomplished by a process which is more complex than a simple distribution between two liquid phases. Anthocyanidin bands on a chromatographic column move more slowly than would be anticipated from direct determinations of partition coefficients and from the theory of partition chromatography as developed by Martin and Synge (18). Adsorption of the anthocyanidins by the silicic acid seems to be the only possible explanation of this phenomenon at present.

The examination of the pigment mixtures of certain grapes and wines by the procedure described in this paper is now in progress.

LITERATURE CITED

(1) Anderson, R. J., et al., J. Biol. Chem., **57**, 795 (1923); **61**, 97, 685 (1924); **80**, 743 (1928); J. Am. Chem. Soc., **48**, 2997 (1926).
(2) Bate-Smith, E. C., Biochem. J., **43**, xlix (1948).
(3) Bate-Smith, E. C., Nature, **161**, 835 (1948).
(4) Bradfield, A. E., and Penney, M., J. Chem. Soc., **1948**, 2251.
(5) Bradley, W., Robinson, R., and Schwartzenbach, G., Ibid., **1930**, 793.
(6) Ibid., p. 814.
(7) Brown, W. L., J. Am. Chem. Soc., **62**, 2808 (1940).
(8) Craig, L. C., J. Biol. Chem., **150**, 33 (1943).
(9) Forsyth, W. G. C., Nature, **164**, 25 (1949).
(10) Genevois, L., and Ribereau-Gayon, J., "Le Vin," Actualités Scientifiques et Industrielles, p. 1017, Nutrition XX, pp. 52–62, Paris, Herman et Cie, 1947.
(11) Gentilini, L., Ann. chim. applicata, **29**, 169 (1939).
(12) Hayashi, K., Phytochim. Acta, **8**, 102 (1934).
(13) Karrer, P., and Strong, F. M., Helv. Chim. Acta, **19**, 25 (1936).
(14) Karrer, P., and Weber, H. M., Ibid., **19**, 1025 (1936).
(15) Karrer, P., and Widmer, R., Ibid., **10**, 16 (1927).
(16) Ibid., pp. 24–5.
(17) Levy, L. F., Posternack, T., and Robinson, R., J. Chem. Soc., **1931**, 2715.
(18) Martin, A. J. P., and Synge, R. L. M., Biochem. J., **35**, 1358 (1941).
(19) Mohler, H., and Hämmerle, W., Z. Untersuch. Lebensm., **70**, 193 (1935).
(20) Robertson, A., and Robinson, R., Biochem. J., **23**, 35 (1929).
(21) Robinson, G. M., and Robinson, R., Ibid., **25**, 1693 (1931).
(22) Ibid., **28**, 1719 (1934).
(23) Spaeth, E. C., and Rosenblatt, D. H., Science, **110**, 258 (1949).
(24) Synge, R. L. M., Analyst, **71**, 256 (1946).
(25) Willstätter, R., and Zollinger, E. H., Ann., **408**, 83 (1915); **412**, 195 (1916).
(26) Ibid., p. 210.

Estimation of Amino Acids and Amines on Paper Chromatograms

RICHARD J. BLOCK

Department of Biochemistry, New York Medical College, New York 29, N. Y.

Detailed directions are presented for the preparation of one- and two-dimensional paper chromatograms for the separation of all the common α-amino acids and a number of nonvolatile amines. The quantity of color, as measured by a simple photoelectric densitometer, developed by ninhydrin or other reagents in each spot on these chromatograms is proportional to the concentration of the substance at that spot. This observation has been used as the basis for estimation of the amino acids in less than 0.1 mg. of casein nitrogen.

SINCE the appearance of the now classical publication of Consden, Gordon, and Martin (7), the use of paper chromatography in almost all phases of biochemical research has reached astonishing proportions. The theory, practice, and extension of this intriguing technique have been summarized in two recent reviews (11, 14). The relative simplicity of qualitative paper chromatography encouraged a number of investigators to attempt to develop it into a quantitative or semiquantitative procedure. These endeavors have led to several satisfactory but relatively tedious methods (2, 11), which are based essentially on matching colors on the paper (12), extraction of the amino acids from the chromatogram and subsequent determination by conventional micromethods (11, 17), determination of the area occupied by a substance on the paper (9, 10), and photoelectric transmission densitometry (2, 3, 5, 13) of the colored substances on the chromatogram.

This paper describes in detail the procedures which, in the author's hands, have given so far the most satisfactory results.

EXPERIMENTAL

Hydrolysis. WITH HYDROCHLORIC ACID. A sample of protein containing 1.60 mg. of nitrogen is hydrolyzed under reflux with 10 ml. of 6 N hydrochloric acid for 20 hours. The excess hydrochloric acid is removed by evaporation to dryness in vacuo at 35° C. or on the steam bath, and the resulting thin film of amino acid hydrochlorides is placed in a vacuum desiccator over soda lime for 24 hours or longer. The hydrolyzate is then taken up in warm water, filtered, again evaporated to dryness, and finally taken up in exactly 1 ml. of 10% isopropyl alcohol. This solvent is used because it is an effective preservative and yet does not cause esterification (4) under these conditions.

WITH HYDROCHLORIC-FORMIC ACID. It has been previously shown that cystine is decomposed when a hydrolyzate is allowed to stand (4). Therefore, fresh hydrolyzates are prepared by the above described procedure or preferably with a 1 to 1 mixture of 6 N hydrochloric acid and 90% formic acid (by volume) as described for the Winterstein-Folin method (8).

WITH SULFURIC ACID. Approximately 10 mg. of protein are hydrolyzed by boiling under reflux with 10 ml. of 8 N sulfuric acid for 20 to 24 hours. Hot saturated barium hydroxide is added to the hydrolyzate until pH 11 or higher is reached. Ammonia is removed by distillation in vacuo (8). The barium ion is then precipitated by the addition of a slight excess of 1 N sulfuric acid, and the precipitated barium sulfate is removed by centrifugation and thoroughly washed with hot water. The filtrate and washings are concentrated to dryness and taken up in 10% isopropyl alcohol.

WITH TRIFLUOROACETIC ACID. The several proteins (casein, lactalbumin, and wheat gluten) which have been tested are satisfactorily hydrolyzed by boiling under reflux with 80% trifluoroacetic acid for 48 hours. At the end of this period, the excess organic acid is removed by extraction with ether. The aqueous layer is evaporated to dryness and taken up in 10% aqueous isopropyl alcohol as described above.

WITH BARIUM HYDROXIDE. A sample of protein containing 1.60 mg. of nitrogen is boiled with 10 ml. of 14% barium hydroxide in an oil bath at 125° C. under reflux for 18 to 20 hours. The barium is removed with a slight excess of 1 N sulfuric acid and the barium sulfate precipitate is thoroughly washed with hot water containing a few drops of acetic acid. The filtrate is concentrated to a small volume in vacuo and then evaporated to dryness in a desiccator over calcium chloride. The residue is taken up in 1 ml. of 10% of isopropyl alcohol.

Preparation of Standard Solutions. All the amino acids or amines to be studied are prepared in 10% isopropyl alcohol at a concentration of 5.0 millimoles per ml. Cystine, tyrosine, and certain other amino acids are dissolved by the addition of a minimal quantity of dilute hydrochloric acid. More dilute standards are prepared from the originals at concentrations of 2.50 and 1.25 millimoles, respectively. Mixtures of compounds may be readily prepared from these standards.
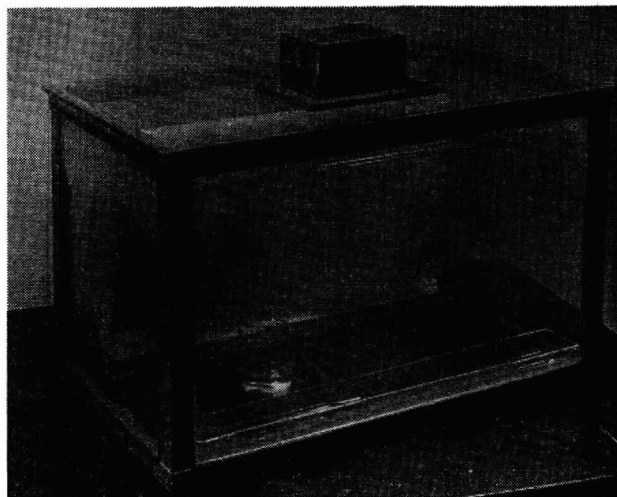

Figure 1. Chromatogram Chamber

Micropipets. Originally micropipets were made in the laboratory, but it was found that those sold by the Microchemical Specialties Company, 1834 University Ave., Berkeley 3, Calif., are entirely satisfactory. The 25μl. pipet calibrated at 5μl. is routinely employed. The solution to be measured is drawn into the pipet, using the usual rubber tubing and glass mouthpiece of a blood pipet. The tip of the pipet is wiped clean with a piece of very soft tissue paper and the solution is brought to the mark by touching it to tissue paper.

Types of Filter Paper. Although Whatman No. 1 paper has been generally employed since the introduction of this technique by Consden *et al.* (*7*), numerous other types have been used (*2, 5, 11, 14*). Considerable success has been achieved in this laboratory with Whatman Nos. 1, 3, and 54 and with S. and S. Nos. 598, 596, and 507. After a great many tests on these and other filter papers, the author prefers Whatman No. 4 for all substances to be identified by the ninhydrin reaction. Standard-size sheets (18.5 × 22.5 inches) are purchased in lots of 100 sheets. Although the great majority of such lots have been satisfactory, one batch of paper gave unsatisfactory chromatograms.
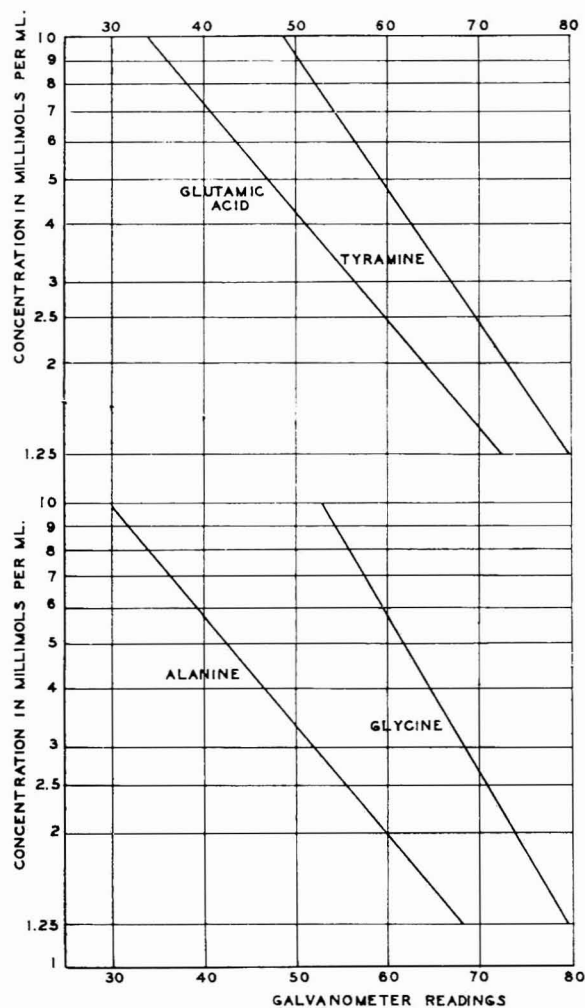

Figure 2. Typical Calibration Curves

Chromatograph Chamber. Rectangular glass aquaria which may be purchased at any pet shop have been used for the past 4 years. Aquaria, 52 cm. long, 32 cm. high, and 26 cm. wide, are generally satisfactory. The lower portion of the sides and all the joints are covered by a layer of paraffin. Two glass rods running the length of the chamber are sealed with paraffin to the sides of the chamber about 5 cm. out and 2 to 3 cm. from the top, and a third rod is sealed half-way between these two and also 2 to 3 cm. from the top. Glass troughs are made by sealing the ends of 2-inch borosilicate glass tubing and cutting longitudinally down the middle. These may be obtained from the Yonkers Laboratory Supply Company, Yonkers, N. Y. The chamber is covered by a heavy glass plate and pressed down to make a seal with a lead weight (Figure 1).

In operation, three sheets of filter paper 17 to 19 inches wide and 19 to 22 inches long are cut, the unknown solution is applied

as drops about 2.5 cm. from the bottom of the sheet and 2 to 3 cm. apart, and the wet spots are dried. The spots are exposed to the vapors of a 2 N ammonium hydroxide solution for solution for 2 minutes. The troughs are filled with from 20 to 50 ml. of developing reagent, depending on the type of filter paper, and the chamber is covered with the heavy glass plate. The solvents climb by capillary action up the paper (ascending chromatography), then past the support rod and down the other side (descending chromatography). The transparency of the chamber permits visualization of the progress of the solvent at all times and without disturbance. The atmosphere within the chamber may be replaced with illuminating gas, nitrogen, etc., as desired and a beaker containing ammonium hydroxide, hydrocyanic acid, diethylamine, etc., may be placed in the central part of the chamber, if necessary. Excess solvent in the glass troughs serves to keep the atmosphere saturated with respect to the reagents and water.

Three 19 × 22 inch sheets can be run in each chamber at the same time—i.e., 66 one-dimensional runs or three two-dimensional runs in each chamber. For two-dimensional runs, the unknown solution is put on the paper at a point 2.5 cm. from each edge, and then carried through the procedure described above.

Chambers of this type have a number of obvious advantages. They are readily available, inexpensive, compact, and easy to store, and may be placed on top of each other. They permit the use of paper strips as well as large sheets.

Somewhat larger aquaria (61 × 41 × 32 cm.) are used for descending chromatography. In this case, the troughs are held near the top of the chamber by three glass rods fastened to the ends of the box with paraffin.

Preparation of Paper for Chromatograms. When one-dimensional chromatograms are to be used, either strips or a sheet of Whatman No. 4, 18.5 × 21 inches, is marked with pencil by a series of dots each at a distance of 2.5 cm. from the bottom of the shorter dimension and 2.5 cm. apart. The concentration of the standard or the designation of the unknown is then written below each with pencil to ensure proper subsequent identification. When two-dimensional chromatograms are to be employed, a line is drawn 2.5 cm. parallel to the edge of the lesser length and a mark is made 2.5 cm. from one edge along this line. This indicates the point of application of the substances to be chromatogrammed.

The papers are then placed on clean glass plates one over the other in echelon, each separated by a glass plate. It is important to hold the papers flat on the glass plates. This is conveniently done by placing the covering plate approximately 1 cm. above the point to which the solution is to be applied and placing glass strips on the lower portion of the paper.

In order to increase the rate of drying of the solution, an infrared lamp is held over the paper. The tip of the micropipet is gently touched to the paper at the designated point at such an angle that the solution is slowly sucked from the pipet by the capillarity of the paper. For best results, only 0.005 ml. of solution should be applied at any one time. If more than this quantity is desired, the paper is thoroughly dried by the "heat lamp" or a blast of warm air, and then a second aliquot is applied. In this way, as much as 0.060 ml. of solution has been put on without increasing the area at the point of application. After the sheets are dry, the papers are placed in the chromatogram chambers.

Probably because these chambers are relatively shallow, it is not necessary to add any of the "inverse" solvent phase (*7*) or to allow the papers to come into equilibrium with the vapors of the solvent before beginning the chromatogram. This is at least true with the solvents to be described.

When two-dimensional chromatograms are run, the order of application of the solvents is of importance. Consden *et al.* (*7*) recommend that the basic solvent (collidine) be run first; Dent (*8*) uses the reverse procedure. The author has employed both methods, but generally prefers the order suggested by Dent (*8*).

If phenol is the first solvent, the chromatogram is started at about 9 A.M. and removed from the chamber 36 hours later (Whatman No. 4 paper) or after 24 hours (S. and S. No. 598 paper). The chromatograms are hung in the hood from a lattice made of string and dried with a current of warm air (30° C.) for 6 hours. An electric timer is useful in shutting off the hood and heating equipment at the proper time. A line 2 to 3 cm. below the line indicating the end of the solvent run is made, and the paper is cut along this line as well as at the line of application of the original mixture. The sheet is then placed in the second solvent

(usually lutidine) and developed for from 20 to 24 hours. The chromatogram is removed, dried in the hood for 30 minutes, and sprayed with the appropriate reagent.

In case the order of use of the solvents is inverted, the same procedure is followed, except that it is unnecessary to dry the lutidine-developed sheets for more than 2 hours before they are ready to be placed in the phenol. As described above, 30-minute drying time is sufficient to remove the excess phenol before spraying when ninhydrin is to be used.

There are two striking differences observed on chromatograms in which the order of solvents is reversed. If phenol is the first solvent, there is almost always a "yellow front" beyond which amino acids and amines do not pass. Unless this yellow front has traveled more than 25 cm., because of insufficient removal of the phenol, poor separation of phenylalanine from the leucines may be encountered. When lutidine is used as the first solvent, this yellow front is not observed, but higher quantities of amino acids are required to give equal color intensities to those found when phenol is the first solvent. When phenol is the second solvent, a much greater range of colors among the various amino acids is observed than by the reverse procedure. This marked difference in the color of various amino acids on the lutidine-phenol chromatograms is in agreement with the report of Consden *et al.* (7).

Solvents. Although approximately 50 different solvents have been investigated, only a few of the most generally useful ones are described here.

PHENOL. One hundred milliliters of distilled, metal-free water are dissolved by warming in 400 ml. of Mallinckrodt Gilt Label liquid phenol, and 20 mg. of 8-quinolinol are then dissolved in the phenol. This solvent is stored in the refrigerator. The cold causes separation into two layers. When the phenol solution is to be used, the bottle is violently shaken and the desired quantity of the emulsion is removed. The phenol is then gently warmed for a few minutes until the water has been completely dissolved. Thirty to 35 ml. of phenol solution are added to each trough.

A beaker containing 30 ml. of 0.3% ammonia is also placed in the chamber (7) in order to neutralize the amino acids. The size of the spots and the location of the basic amino acids are markedly influenced by the presence of ammonia vapors.
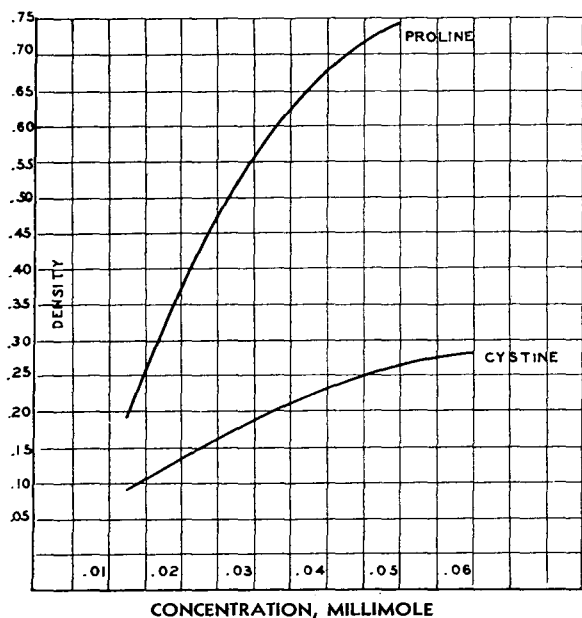

CONCENTRATION, MILLIMOLE

Figure 3. Plots of Color Density

If the addition of 8-quinolinol (8-hydroxyquinoline) is not desired, it is necessary to place a small beaker containing approximately 100 mg. of sodium cyanide in 4 to 6 ml. of water in the chamber. The air in the chamber is replaced with illuminating gas after the papers have been introduced.

Because the laboratory is usually below 23° C., the commer-

cially available "electric blankets" are suitable for keeping the temperature satisfactorily constant. Four chambers may be completely wrapped in a blanket of the size sold to cover a double bed. (If the temperature is above 25° C. during development with phenol, part or all of the methionine is oxized to methionine sulfoxide.)

LUTIDINE. A mixture of 55 volumes of 2,6-lutidine (Matheson Company, East Rutherford, N. J.), 20 volumes of alcohol (ethyl alcohol, isopropyl alcohol, *tert*-butyl alcohol, or *tert*-amyl alcohol), and 25 volumes of water is used as the principal "basic" solvent. To every 500 ml. of this solvent, 3.3 ml. of diethylamine (Sharples Chemical Company, Philadelphia) are added. A beaker containing approximately 100 mg. of sodum cyanide in 4 to 6 ml. of water is placed in the chamber (4).

BUTANOL–ACETIC ACID. Ninety volumes of *n*-butyl alcohol and 10 volumes of glacial acetic acid are mixed and the mixture is saturated with water. This solvent is generally used without the presence of a volatile alkali in the chamber, but 0.3%, or 3.0% of ammonia, or 3 ml. of 33% diethylamine may be used for "tightening" the spots of faster moving substances.

sec-tert-BUTYL ALCOHOL. A very useful solvent for the separation of sulfur containing amino acids and peptides (especially glutathione) is prepared from 40 volumes of *sec*-butyl alcohol, 10 volumes of *tert*-butyl alcohol, and 30 volumes of water. Thirty-three per cent diethylamine is usually placed in the chamber with this solvent.

tert-BUTYL ALCOHOL. The platinic iodide test (16) cannot be applied to chromatograms run with secondary-tertiary butyl alcohol unless all the developing solvent is removed with acetone and ether (v/v). If 70% *tert*-butyl alcohol is used instead, the subsequent treatment with acetone and ether is unnecessary.

tert-BUTYL ALCOHOL–FORMIC ACID. A mixture of 15 volumes of 90% of formic acid, 15 volumes of water, and 70 volumes of *tert*-butyl alcohol forms an excellent developing agent for the separation of amino acids into groups from which the individual components are to be determined by the use of other solvents. S. and S. filter paper No. 598 appears to be the paper of choice with this solvent.

Length of Run. Although the time required to develop the chromatogram will depend on the solvent used and the degree of separation desired, the solvent is usually allowed to travel 40 to 50 cm. beyond the point of application of the substances.

Color Development. At the completion of the chromatogram, the papers are removed and dried in a hood with a good draft. If the laboratory temperature is below 30° C., the incoming air is raised to that temperature by an electric radiator (Edwin Wiegand Company, Bloomfield, N. J.), and the papers are dried until little or no solvent odor is detected. Time for the completion of drying depends on the volatility of the solvent, type of paper, etc.

NINHYDRIN. This general reagent for amino acids and primary amines is used in 0.1 or 0.2% concentration in 90% isopropyl alcohol alone or with 1% of acetic acid or 0.004% of stannous chloride (11, 14). The ninhydrin solution is applied evenly over the paper by a DeVilbis spray gun. A Presso-Vac pump (Central Scientific Company, Chicago) makes a convenient source of compressed air. The sprayed sheets are dried at 30° C. for 30 to 40 minutes by a blast of warm air in the hood. If the moisture of the laboratory is low, a steam pot is placed in the hood, with the draft off, and the chromatograms are steamed for 3 to 5 minutes. The steam is blown out of the hood and the chromatograms are allowed to remain at room temperature for from 18 to 28 hours.

PLATINIC IODIDE. The sulfur-containing compounds, cystine, methionine, methionine sulfoxide, and glutathione are readily separated from each other on Whatman No. 4 or S. and S. No. 598 filter papers with 70% *tert*-butyl alcohol in the presence of diethylamine. If glutathione is not present, butanol–acetic acid becomes a very satisfactory solvent. After removal of the excess solvent from the paper by a blast of warm air, the papers are sprayed with or quickly dipped into the platinic iodide reagent of Winegard *et al.* (16). The chromatograms are partially dried in the hood for 5 minutes. The damp chromatograms are then placed in a chamber which contains a beaker of concentrated hydrochloric acid. The presence of S^{++} or S^{++++} compounds is indicated by a bleached area against a purple-red background. When this has taken place the chromatograms are dried, and the concentration of each substance is calculated from the bleached area according to Fisher *et al.* (9, 10).

PHOSPHO-18-TUNGSTIC ACID. The Winterstein-Folin method for cystine estimation (4, 18) is applied to chromatograms in the following fashion: Ten milliliters of Folin's phospho-18-tungstic acid and 10 ml. of water are added to 25 ml. of a saturated solu-

tion of sodium bicarbonate. Then 5 ml. of a freshly prepared 10% solution of sodium sulfite are added with mixing and the papers, which have been previously dried as described above, are immediately sprayed or dipped. An area of blue, which indicates the presence of cystine or other reducing material, is seen on the paper immediately or within a few moments.

DIAZOTIZED SULFANILAMIDE. This reagent, which is used to determine histidine, histamine, tyrosine, and tyramine, has been described in detail recently (2).

ISATIN. Acher, Fromageot, and Jutisz (1) have found that 0.2% of isatin in n-butyl alcohol containing 4% (by volume) of acetic acid will give only blue or bluish-green spots with proline and hydroxyproline. Butanol-acetic acid gives good separations of these two amino acids. After the removal of the excess developing solvent, the chromatogram is sprayed with the isatin reagent and the sheets are dried in the hood. The color is allowed to develop at room temperature or the chromatograms may be heated in an oven at 75° C. for 15 minutes. If heat is employed, the blue color of proline and hydroxyproline develops, but a number of other amino acids show weak red or pink spots.

p-DIMETHYLAMINOBENZALDEHYDE. Tryptophan and other indole derivatives will give a blue color on paper chromatograms when treated with 1% of p-dimethylaminobenzaldehyde in approximately 3.7% of aqueous hydrochloric acid. Numerous other aldehydes may be substituted for Ehrlich's reagent (4). Butanol-acetic acid or other solvents may be used for development of the chromatogram. The papers, after being sprayed with Ehrlich's reagent, are dried in a current of warm air. The color densities should be read as soon as the chromatograms are dried.

Athough a number of other specific color tests are available for use with paper chromatograms (phosphomolybdotungstic acid for phenols and indoles; Sakaguchi's test for guanido groups; o-phthaldialdehyde for glycine, etc., 4), the reagents described above are the only ones that have been studied to date for their application to quantitative chromatography.

Measurement of Color Density. Although a suitable source of light of constant luminosity is easy to construct, the light source sold by the Photovolt Corporation, New York, N. Y., has proved to be very satisfactory. A standard photocell and a Rubicon Spotlight galvanometer (No. 3402-HH) or a Pfaltz and Bauer multiple mirror galvanometer (No. 1810) are all the equipment required for the determination of the color density. A photoelectric transmission densitometer (W. M. Welch Mfg. Company, 1515 Sedgwick St., Chicago 10) has also given excellent results. The light is circumscribed by means of a 15.6 sq. mm. round disk or a 5 × 5 mm. square disk. A 570-mm. filter between light source and the photocell may be used but it is not necessary. A piece of filter paper, identical to that used for the chromatogram, is employed to adjust the instrument to 100% of light transmission.

When one-dimensional chromatograms are used, the strip of paper is pulled slowly, by a small constant-speed motor, across the beam of light and the deflection of the galvanometer is noted at each peak. In this way a paper strip 20 mm. long containing seven amino acid spots, well separated from each other, may be easily scanned in 2.5 minutes.

RESULTS

One-Dimensional Chromatograms. When the spots are clearly separated and have sharp distinct boundaries, the area method of Fisher et al. (9, 10) gives very satisfactory results. However, if these conditions are not fulfilled, advantage is taken of the observation that the maximum color density of each spot is proportional to the concentration of material in the entire spot. The evidence for this observation was initially made by inspection of the essentially triangular nature of the color density curves which were obtained by plotting the color density observed against the distance from the point of application (3, 5). The fact that such a relationship is not unlikely may be deduced from the liquid-liquid distribution diagrams of Craig and others (6, 11, 14, 15).

When the maximum color density was plotted against the concentration of amino acid or amine, a relationship was found over the range of 0.005 to 0.050 millimole of amino acid or amine. Figure 2 gives a few typical calibration curves, but similar results have been obtained with all amino acids and amines which give a ninhydrin, Pauly, or Voisenet test. The reagents for proline (isatin) and for cystine (phosphotungstic acid) give the types of curves shown in Figure 3.

The proportionality of maximum color to concentration was observed only when aliquots of equal size were applied to the paper. If this precaution was not observed, there were marked differences in the areas of the resulting spots on the chromatogram and in the intensity of color at the maximum. However, even in these cases, there is a direct proportion between the concentration of material and the product of the area of the spot times the maximum color density (2, 3). Because of the added labor needed in this type of estimation, it has now been largely abandoned but it is very useful when "tight" spots cannot be obtained.

Because of the number of factors which are practically beyond control in paper chromatography, it is advisable to run five or more unknown replicates and an equal number of standard mixtures on the same sheet. Recovery experiments on standard solutions added to the unknown mixture also often facilitate interpretation and calculation of the results.

A list of the amino acids, together with suggested solvents and color reagents, which are readily estimated on one-dimensional chromatograms, is given in Table I. This list is not complete, as other amino acids may be sufficiently well separated for quantitative estimation on one-dimensional chromatograms by the use of other solvents. However, the amino acids listed in Table I have been found repeatedly to give satisfactory results under the conditions mentioned.

Because one-dimensional chromatography offers a number of advantages over two-dimensional paper chromatograms, including controls on the same sheet, increased numbers of replicate analyses, ease of operation, etc., two one-dimensional chromatograms with appropriate solvents have proved to be particularly advantageous in several instances.

A group separation is made by placing forty 0.005-ml. aliquots of a protein hydrolyzate which contains the equivalent of 25 mg. of protein per ml. along a line 2.5 cm. from the bottom of two 18 × 21 inch sheets of S. and S. 598 filter paper, each spot being about 12 mm. from the next. The chromatograms are then developed

Table I. Procedures for Estimating Amino Acids and Glutathione on One-Dimensional Paper Chromatograms

Amino Acid	Solvent	Color Reagent	Method of Determination
Aspartic acid	Phenol–NH₃	Ninhydrin	Color density
Glutamic acid	Phenol–NH₃	Ninhydrin	Color density
Serine	Phenol–NH₃	Ninhydrin	Color density
Glycine	Phenol–NH₃	Ninhydrin	Color density
Threonine	Phenol–NH₃	Ninhydrin	Color density
Alanine	Phenol–NH₃	Ninhydrin	Color density
Valine	Lutidine-alcohol-(C₂H₅)₂ NH	Ninhydrin	Color density
Leucine	Lutidine-alcohol-(C₂H₅)₂ NH	Ninhydrin	Color density
Isoleucine	Lutidine-alcohol (C₂H₅)₂ NH	Ninhydrin	Color density
Phenylalanine	Lutidine-alcohol-(C₂H₅)₂ NH	Nihydrin	Color density
Glutathione	tert-Butyl alcohol	H₂PtI₆	Area (9,10)
Cystine and cysteine	tert-Butyl alcohol	H₂PtI₆	Area
Methionine and methionine sulfoxide	tert-Butyl alcohol	H₂PtI₆	Area
Proline	Butanol–acetic acid	Isatin	Color density or area
Hydroxyproline	Butanol–acetic acid	Isatin	Color density or area
Histidine and histamine	Butanol–acetic acid	Pauly reagent	Color density
Tyrosine and tyramine	Butanol-acetic acid	Pauly reagent	Color density
Tryptophan	Butanol–acetic acid	Voisinet reagents	Color density or area

with a solvent—e.g., lutidine–ethyl alcohol–water. At the completion of the development with this solvent, three 10-mm. guide strips are cut, one at each end and one from the center of the sheets. These are dipped into the ninhydrin solution and the results are used to guide the horizontal cutting of the chromatogram. In this way, the following groups are obtained starting from the base line: (a) aspartic acid, glutamic acid, lysine, and arginine; (b) histidine, glycine, serine, hydroxyproline, alanine, proline, and threonine; (c) valine, methionine, isoleucine, leucine; (d) tyrosine and phenylalanine. The separate strips are thoroughly extracted with hot water, the extracts are concentrated to dryness in vacuo, and the residues are dissolved in 1 ml. of 10% isopropyl alcohol. The amino acids in these groups are readily separated on one-dimensional chromatograms as described above (cf. 4, 7 for other solvents).

This procedure seems to be the method of choice, at the present time, when a number of proteins are to be analyzed simultaneously. Where a recording galvanometer is available for use with the photoelectric densitometer, a large number of determinations may be carried out with a minimum of labor. Although this method has not been investigated as thoroughly as the other procedures given in this paper, it appears that the standard amino acid solutions should be put through the same treatment as the unknown solutions and at the same time.

The relationship between color density and concentration of material on a chromatogram appears to be valid, even though not all of the substance may react with the color reagent, or more than one colored compound is formed by the reaction between the substance and the color reagent (17).

Two-Dimensional Chromatograms. In contrast to the degree of separation which may be achieved on one-dimensional chromatograms, all the amino acids commonly present in an acid hydrolyzate of a protein may be well separated from the rest by phenol and lutidine two-dimensional chromatograms, except for leucine and isoleucine which appear as one spot with lutidine–ethyl alcohol and as two spots with lutidine–amyl alcohol. The usefulness of the two-dimensional chromatogram led the author to investigate the possibility of employing it for the quantitative estimation of amino acids and amines.

FIRST METHOD. A procedure has been described (2) which allows the molecular proportions of each amino acid to the others to be calculated on two-dimensional chromatograms. This technique was developed from the observation that although the color density of any one amino acid may vary from day to day, probably because of uncontrollable laboratory conditions such as temperature, contaminants in the atmosphere, etc., the amount of color developed by any amino acid remained proportionally constant to the others on the same chromatogram and to its counterpart on other sheets run the same day.

Table II gives the so-called "standard color ratios" of four concentrations of mixtures of amino acids on phenol-lutidine two-dimensional chromatograms on Whatman No. 4 paper. There is no significant difference between the color ratios at any of the four concentrations. The standard errors are calculated from eight or nine chromatograms on each level carried out over a 2-week period. These chromatograms were developed with 0.1% of ninhydrin in 90% isopropyl alcohol which contained approximately 4 mg. of stannous chloride per 100 ml. These standard color ratios are not the same as those reported previously (2). In the latter case S. and S. 596 paper sprayed with 0.1 or 0.2% of

Table II. Color Ratios of Amino Acids on Whatman No. 4 Paper Two-Dimensional Chromatograms

	Concentration, Millimole				Mean Color Ratio
	0.00625	0.0125	0.0250	0.0500	
			No. of Runs		
	8	8	9	8	33
Arginine	0.80 ± 0.05	0.77 ± 0.04	0.79 ± 0.08	0.83 ± 0.05	0.80 ± 0.06
Histidine	0.79 ± 0.07	0.78 ± 0.04	0.72 ± 0.07	0.89 ± 0.04	0.85 ± 0.08
Lysine	0.73 ± 0.07	0.70 ± 0.04	0.75 ± 0.05	0.74 ± 0.03	0.73 ± 0.06
Tyrosine	1.02 ± 0.12	1.04 ± 0.07	1.00 ± 0.07	0.99 ± 0.05	1.01 ± 0.03
Phenylalanine	0.97 ± 0.08	0.98 ± 0.07	0.88 ± 0.08	0.90 ± 0.08	0.93 ± 0.08
Methionine	1.05 ± 0.06	1.14 ± 0.08	1.12 ± 0.05	1.13 ± 0.11	1.11 ± 0.08
Serine	1.08 ± 0.05	1.08 ± 0.09	1.10 ± 0.07	1.05 ± 0.08	1.08 ± 0.07
Threonine	0.86 ± 0.05	0.78 ± 0.03	0.89 ± 0.08	0.94 ± 0.04	0.87 ± 0.04
Leucine[a]	1.40 ± 0.13	1.51 ± 0.13	1.48 ± 0.05	1.39 ± 0.03	1.45 ± 0.09
Valine	0.95 ± 0.08	1.05 ± 0.05	1.06 ± 0.07	1.12 ± 0.07	1.05 ± 0.07
Glycine	1.05 ± 0.07	1.00 ± 0.05	1.04 ± 0.12	1.06 ± 0.08	1.04 ± 0.08
Alanine	0.96 ± 0.05	0.94 ± 0.04	1.05 ± 0.08	1.02 ± 0.04	1.00 ± 0.05
Glutamic acid	1.33 ± 0.16	1.33 ± 0.15	1.30 ± 0.08	1.20 ± 0.08	1.29 ± 0.14
Aspartic acid	1.04 ± 0.08	0.91 ± 0.12	0.78 ± 0.08	0.80 ± 0.06	0.87 ± 0.10

Standard error indicated by ±.

[a] Equimolar mixture of leucine and isoleucine developed with phenol and lutidine; consequently molar concentration is twice that of other amino acids.

Table III. Amino Acids in Casein Determined on Two-Dimensional Chromatograms[a]

(Millimoles per 1.60 mg. of N)

Amino Acid	Color Ratio and Standard Mixture	Calibration Curves and Standard Mixture	Color Ratio Alone (2)	Calculated (4)
Arginine	2.45 ± 0.19	2.5	2.4	2.4
Histidine	2.24 ± 0.16	2.2	1.9	2.1
Lysine	5.94 ± 0.33	5.3	5.4	5.8
Tyrosine	4.16 ± 0.08	4.0	3.8	3.8
Phenylalanine	3.85 ± 0.18	3.4	3.6	3.1
Methionine	2.63 ± 0.25	2.7	2.3	2.3
Serine	5.50 ± 0.11	5.5	5.0	5.6
Threonine	3.96 ± 0.28	4.0	3.8	4.0
Leucine	11.70 ± 0.26	11.4	13.5	12.8
Valine	6.11 ± 0.28	5.3	5.7	6.3
Glycine	2.52 ± 0.15	2.4	3.1	2.8
Alanine	3.82 ± 0.07	3.8	3.7	3.7
Glutamic acid	16.0 ± 0.64	15.6	15.3	16.1
Aspartic acid	5.01 ± 0.22	6.1	4.3	5.6
Proline	..	11.2	..	10.5
Hydroxyproline	..	0.0	..	..
No. of chromatograms	8	8	25	

Standard error indicated by ±.

[a] Except proline and hydroxyproline.

ninhydrin in water-saturated butyl alcohol was used. It is advisable to carry out five replicate unknown and standard control chromatograms simultaneously. Then, if the molecular ratios of the amino acids in the unknown are within the range 1.25 to 10.0 millimole of amino acid per ml. of solution, a reasonable approximation of its relative composition may be achieved on five or ten chromatograms as compared to the considerably larger number reported previously (2). A closer approximation to the true composition is then obtained by the procedure described below, although absolute values may be calculated from two-dimensional chromatograms by the "color ratio" method as described previously (2) from data obtained on one-dimensional chromatograms.

SECOND METHOD. This procedure is essentially the same as that used on one-dimensional chromatograms and is based on the experimental observation that the maximum color density is a function of the concentration of the amino acid. Color density plots of these data are very similar to those obtained with one-dimensional chromatograms (Figure 2).

This procedure consists in carrying out a number of replicate two-dimensional chromatograms on the unknown and on the three standard known mixtures simultaneously. Calibration curves are drawn from the results obtained with the known mixtures and the quantities of each unknown are estimated from these curves. The curves should not be extrapolated beyond actual experimental findings. If the intensities of the color of the spots of the unknown solution are not within the standard range, this should be corrected by proper concentration or dilution of the

unknown solution. In practice, amino acids which are most likely to be off the calibration curves (glutamic acid, glycine, methionine, etc.) are readily estimated on one-dimensional chromatograms.

After an approximate idea of the composition of the unknown solution has been attained by either of the above methods, a mixture of standard amino acids is prepared which contains the same molar concentration of each substance as is estimated to be present in the unknown solution. A number of replicate chromatograms are then carried out simultaneously on this standard mixture and on the unknown. The results of two separate experiments based upon the color ratio method and on calibration curves, respectively, are given in Table III, together with earlier results (2) obtained from many more chromatograms using the color ratio method described above.

A similar proportionality between color density and concentration has been found on chromatograms of histamine, cadaverine, putrescine, tyramine, phenylethylamine, propylamine, isobutylamine, and n-amylamine. The amines were separated from amino acids and peptides by extraction with ether in a continuous extractor from a dilute alkaline solution.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Acher, R., Fromageot, C., and Jutisz, M., *Biochem. et Biophys. Acta*, 5, 81 (1950).
(2) Block, R. J., *Proc. Soc. Exptl. Biol. Med.*, 72, 337 (1949).
(3) Block, R. J., *Science*, 108, 608 (1948).
(4) Block, R. J., and Bolling, D., "Amino Acid Composition of Proteins and Foods," 2nd ed., Springfield, Ill., C. C Thomas, 1950.
(5) Bull, H. B., Hahn, J. W., and Baptist, V. H., *J. Am. Chem. Soc.*, 71, 551 (1949).
(6) Bush, M. T., and Densen, P. M., ANAL. CHEM., 20, 121 (1948).
(7) Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, 38, 224 (1944).
(8) Dent, C. E., *Ibid.*, 43, 169 (1948).
(9) Fisher, R. B., Parsons, D. S., and Holmes, R., *Nature*, 164, 183 (1949).
(10) Fisher, R. B., Parsons, D. S., and Morrison, S. A., *Ibid.*, 161, 764 (1948).
(11) Martin, A. J. P., *Ann. Repts. on Progress Chem. (Chem. Soc. London)*, 45, 267 (1949).
(12) Polson, A., *Biochim. et Biophys. Acta*, 2, 575 (1948).
(13) Rockland, L. B., and Dunn, M. S., *J. Am. Chem. Soc.*, 71, 4121 (1949).
(14) Williams, R. T., and Synge, R. L. M., "Partition Chromatography," Biochemical Society Symposia No. 3, Cambridge, Cambridge University Press, 1949.
(15) Williamson, B., and Craig, L. C., *J. Biol. Chem.*, 168, 687 (1947).
(16) Winegard, H. M., Toennies, G., and Block, R. J., *Science*, 108, 506 (1948).
(17) Woiwod, A. J., *Biochem. J.*, 45, 412 (1949).
(18) Work, E., *Lancet*, 256, 652 (1949).

Microdetermination of Sulfur by the Grote Method

Photometric Detection of the Titrimetric End Point

ROLAND N. WALTER

Hercules Experiment Station, Hercules Powder Company, Wilmington 99, Del.

In the Grote combustion micromethod for determining sulfur in organic compounds, the sulfate formed is usually titrated with standard barium chloride solution, using tetrahydroxyquinone (THQ) or an alkali salt of rhodizonic acid as an internal indicator. A photometric method of detecting the end point, which is more objective than the usual visual method, is described. Titration results are reproducible within 0.004 mg. of sulfur.

FOR the determination of sulfur in small samples of organic compounds, a microprocedure must be used. The recent literature on this subject has been well summarized by Willits (6), who, with Ogg and Cooper (2), described a visual titrimetric technique for the identification of the end point in the volumetric determination of soluble sulfates with barium chloride and dipotassium rhodizonate. Hallett and Kuipers (1) had previously used a somewhat similar technique. Steyermark, Bass, and Littman (4) have applied the titrimetric technique of Ogg et al. (2) to the analysis of organic compounds decomposed by the Carius method.

A study of the transmittance curves of disodium rhodizonate and of tetrahydroxyquinone (THQ) at the end point showed that the change in color at this point is small. Accordingly, in order to have a more objective method of identifying the end point, and because of the experience of others in this laboratory with the use of photoelectric photometers (3) in detecting the exact end points of titrations, the writer has made use of these instruments in the present work.

The spectral characteristics of tetrahydroxyquinone and disodium rhodizonate indicators were obtained by means of a General Electric recording spectrophotometer. Figures 1 and 2 show transmittance curves with potassium sulfate and indicator, and curves with potassium sulfate, indicator, and a 5% excess of 0.02 N barium chloride. The two sets of curves are very similar. Because at the end point a change in hue takes place, the wave length at which the greatest change in transmittance occurs is not necessarily at the absorption maximum (480 mμ). Actually it occurs at a point between 520 and 530 mμ. At the absorption maximum, the transmittance increases with addition of barium chloride, while it decreases at wave lengths above 500 mμ.

In the early experimental work, a specially designed Hercules general-purpose photometer and a tristimulus blue filter (Henry A. Gardner Laboratory) with a transmittance maximum at 440 mμ were used. Later a Klett-Summerson photoelectric colorimeter and a Baird interference filter having a transmittance maximum at 537 mμ were found to be equally suitable. With the latter combination it was possible to identify the end point by

the deflection of the galvanometer needle, thus eliminating the necessity of plotting a curve. This new technique is advantageous when the method is used routinely.

APPARATUS

Klett-Summerson photoelectric colorimeter.

Green interference filter with a transmittance maximum at 520 to 540 mμ.

Titration cell (Figure 3).

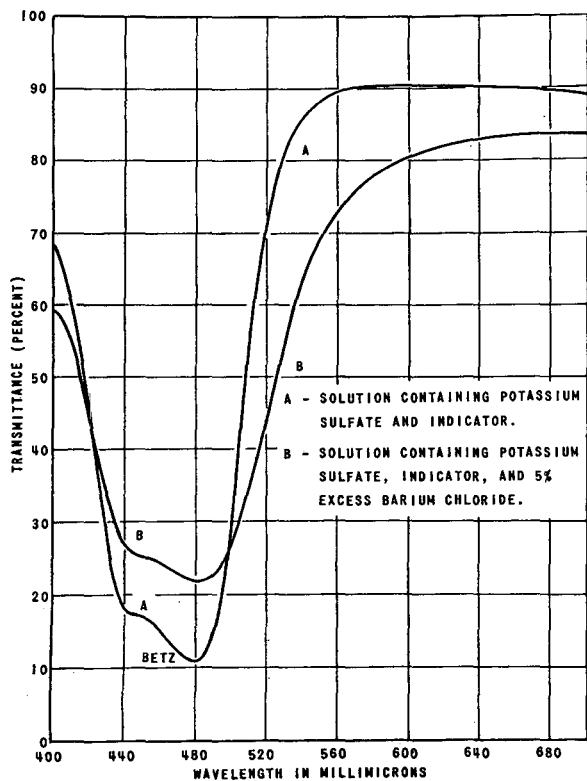A three-hole No. 6 rubber stopper used to close the titration cell and to hold the stirrer shaft and buret tip.

Figure 1. Spectral Transmittance Curves for THQ Indicator (Betz)

A mechanical stirrer consisting of a small electric motor and shaft. As a propeller, diagonal grooves are cut in the lower half of a No. 5 rubber stopper.

A 5-ml. buret graduated in 0.01-ml. divisions, with interchangeable tip. For flexibility in operation, an auxiliary buret tip is inserted through the rubber stopper and connected to the buret by means of a 6-inch piece of Tygon tubing.

REAGENTS

Potassium sulfate solution, 0.02 N, prepared from dried A.C.S.-grade material.

Barium chloride solution, 0.02 N, prepared and standardized against the 0.02 N potassium sulfate solution, using the procedure described subsequently for the titration of samples.

Nitric acid solution, approximately 0.1 N.

Sodium hydroxide solution, approximately 0.1 N.

Indicator. The prepared THQ indicator sold by Betz Laboratories, Philadelphia, Pa., is already diluted with an inert material. The pure disodium rhodizonate sold by Smith-New York, Inc., Freeport, Long Island, N. Y., must be diluted 1 to 300 with powdered sucrose.

PROCEDURE

The combustion of the organic sample is carried out according to the method of Sundberg and Royer (5). The solution of combustion products in 0.1 N sodium hydroxide is washed from the Grote absorber and vapor trap with a minimum amount of distilled water into a 125-ml. Erlenmeyer flask, and 5 ml. of

saturated bromine water are added to oxidize any sulfite to sulfate. An amount of 0.1 N nitric acid slightly greater than the volume of 0.1 N sodium hydroxide used at first is then added. The solution is boiled until the excess bromine is dispelled and the volume is reduced to about 15 ml.

After the solution has been cooled and treated with 2 drops of 1% phenolphthalein solution, it is made slightly alkaline with 0.1 N sodium hydroxide and then faintly acid with 0.01 N nitric acid. This solution is transferred to the photometer titration cell, and an equal volume of alcohol and about 0.15 gram of indicator are added.

The green interference filter is placed in the Klett-Summerson photoelectric colorimeter, the titration cell is inserted, and the cell is closed with the three-hole No. 6 rubber stopper which has the mechanical stirrer shaft extending through the center hole. The height of the stirrer is adjusted so that the rubber blades fall in the center of the bulb portion of the cell. The stirrer is run at a moderate speed. The offset buret tip is inserted through one of the remaining holes until its end is below the surface of the solution. Next, the density dial of the photometer is set at zero and the galvanometer adjusted to zero current by means of the null control which is connected to the balancing photocell. The barium chloride solution is now added at a rate of about 3 ml. per minute. When about two thirds of the required amount has been added, there will be a considerable deflection of the galvanometer needle. This is caused by the temporary formation of the red-colored barium salt of the indicator, which decreases the transmission of the solution. When the needle has been deflected a distance corresponding to 30 or 40 dial units, the addition of barium chloride is stopped for 5 to 10 seconds, to allow the pseudo end point to disappear. As soon as the needle returns to a stable deflection (not necessarily zero), the addition of titrant is resumed in 0.05-ml. portions. In order to keep the galvanometer needle on the scale, it is necessary to turn the density dial slightly from time to time; this should be done after the needle has come to a stable position.
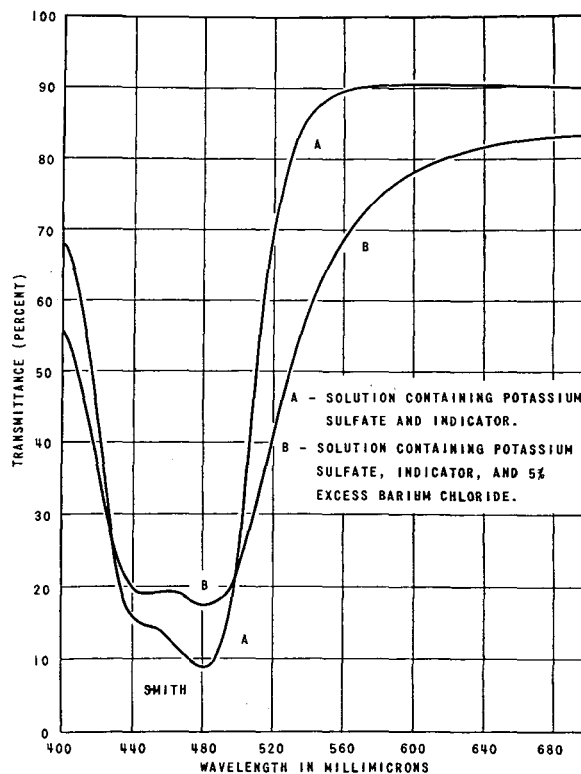

Figure 2. Spectral Transmittance Curves for Disodium Rhodizonate Indicator (Smith-New York)

When the end point is imminent, the concentration of the solution in terms of sulfate has become small, and the time required to dispel the pseudo end point becomes longer. As soon as the galvanometer needle becomes sluggish in its return to a stable position, the barium chloride additions are decreased to 0.02-ml. portions.

The end point has been reached when, upon addition of 0.02 ml. of barium chloride, no backward movement of the needle is noted after its deflection to the right.

In accordance with the procedure of Ogg et al. (2), enough additional standard potassium sulfate solution is added before the beginning of the titration, if necessary, so that at least 3 ml. of barium chloride solution are required. The reason for this step is that the indicator apparently does not have enough barium sulfate surface upon which to absorb unless this much barium chloride is used.

DISCUSSION

In the early work on the titration of small amounts of sulfate, using the Hercules general-purpose photometer equipped with a blue tristimulus filter, it was possible to detect the end point merely by observation of the galvanometer needle deflection. Unfortunately, the Klett-Summerson photometer and blue filter combination were found to be too insensitive to detect the end point by this technique. However, by plotting the titration data to locate the end point, highly reproducible results were obtained. A typical titration curve is shown in Figure 4.
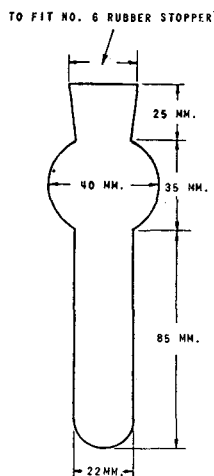

Figure 3. Titration Cell for Use with Klett - Summerson Photometer

Table I. Titration of Known Amounts of Potassium Sulfate

(Using Klett-Summerson photometer and green filter)

S Present, Mg.	S Found, Mg.
1.234	1.233
1.234	1.230
1.234	1.234
1.234	1.233
1.851	1.847
1.851	1.850
1.851	1.850
3.085	3.082

Additional tests were made with the Klett-Summerson photometer, substituting a green interference filter for the blue filter. This new combination proved to be sufficiently sensitive for detection of the end point by observing the deflection of the galvanometer needle, as before. The results of titrations of known

Table II. Comparison of Methods for Determination of Sulfur in Known Compounds

	Sulfur Found, %			
	Hercules general-purpose photometer	Klett-Summerson photoelectric colorimeter	Parr bomb	Sulfur Calculated, %
Cystine	26.69 26.73 26.75 26.99	26.82	...	26.69
2,4-Dichloro-phenyl-4-toluene sulfonate	10.09 10.35	...	10.00 10.20 10.28 10.28	10.11
Sulfonal	27.95 28.21	...	28.4 28.2 28.6	28.09
S-Benzyl-thiuronium chloride	...	15.86 15.77	...	15.82

small quantities of potassium sulfate are shown in Table I and indicate an accuracy and precision of at least 0.004 mg. of sulfur.

Table II contains the results found on determining sulfur in four compounds by the foregoing procedure. In this work both the Hercules general-purpose photometer and the Klett-Summerson photoelectric colorimeter were used. The Parr bomb results were obtained gravimetrically.

SUMMARY

A photometric method of detecting the end point in the titration of the sulfate formed in the microdetermination of sulfur by the Grote method is described. The proposed method does not require the construction of a curve. It is less subjective than the usual visual method and should assist in overcoming objections to the use of an internal indicator such as tetrahydroxyquinone.
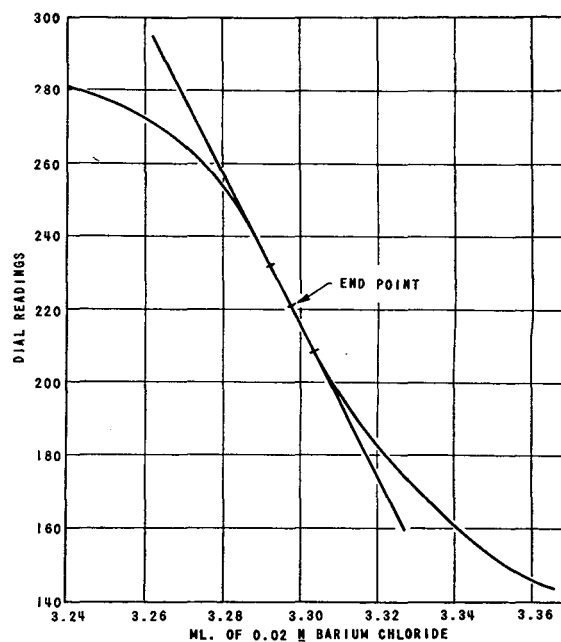

Figure 4. Titration Curve Showing End Point Using Klett-Summerson Photometer with Blue Filter

This technique should be applicable when other makes of photoelectric photometers or spectrophotometers are used, and with any method for determining sulfur in which the sulfur is oxidized to sulfate, such as in the Carius tube, peroxide bomb, or oxygen bomb.

LITERATURE CITED

(1) Hallett, L. T., and Kuipers, J. W., IND. ENG. CHEM., ANAL. ED., 12, 360 (1940).

(2) Ogg, C. L., Willits, C. O., and Cooper, F. G., ANAL. CHEM., 20, 83 (1948).

(3) Osborn, R. H., Elliott, J. H., and Martin, A. F., IND. ENG. CHEM., ANAL. ED., 15, 642 (1943).

(4) Steyermark, Al, Bass, E., and Littman, B., ANAL. CHEM., 20, 587 (1948).

(5) Sundberg, O. E., and Royer, G. L., IND. ENG. CHEM., ANAL. ED., 18, 719 (1946).

(6) Willits, C. O., ANAL. CHEM., 21, 136 (1949).

Converting Platinum Resistance to Temperature

D. N. EGGENBERGER, *Armour and Company, Chicago, Ill.*

THE usual method of converting platinum resistance thermometer readings to corresponding temperatures involves direct substitution into the familiar Callendar formula and application of the δ- and β-term corrections from tables. Where many series of readings are made, the several minutes required for each conversion result in rather lengthy computations. One obvious means of simplifying conversions would be to use direct-reading graphs. But because the resistance thermometer is sensitive to 0.001° C., an inordinate number of such graphs would be required to utilize the full capabilities of the apparatus—for example, at 1 mm. per 0.001° C. a chart 1 meter square would be required for every degree of temperature range.

A convenient compromise between these extreme methods has been developed for a nominal 25-ohm resistance-thermometer. The method takes advantage of the facts that the resistance-temperature relation is not far from linear and that for such a thermometer 1 ohm of resistance change corresponds roughly to 10 Celsius degrees of temperature change. The process of conversion then involves the following steps: (1) Find in a table the temperature corresponding to the integral part of the resistance, (2) add ten times the decimal part of the resistance, and (3) add the parabolic correction taken from a graph. For temperatures below zero, the parabolic-correction graph includes the β-term of the Van Dusen equation.

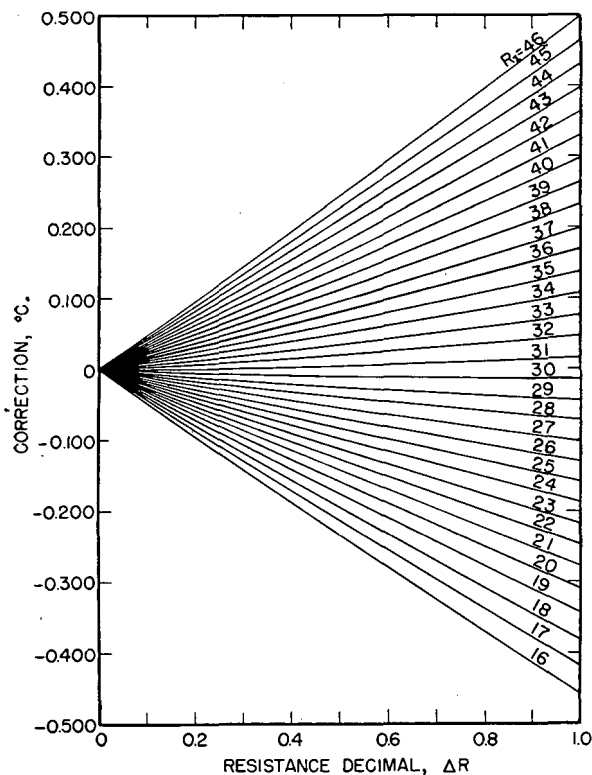The labor of preparing data for constructing the correction graph can be reduced by using the following development.

Figure 1. Parabolic-Correction Curves

The Callendar equation

$$t = 100 \frac{R_t - R_0}{R_{100} - R_0} + \delta \left(\frac{t}{100} - 1\right) \frac{t}{100}$$

can be readily rearranged into a form explicit in t:

$$t = A - B\sqrt{C - R_t} \qquad (1)$$

where $A = 50 + \dfrac{5000}{\delta}$, $B = \dfrac{50}{\delta}\sqrt{\dfrac{400\delta}{R_{100} - R_0}}$, and

$$C = (R_{100} - R_0)\left(\frac{\delta}{400} + \frac{1}{2} + \frac{25}{\delta}\right) + R_0$$

Let $S = C - R_I$, where R_I is the integral part of the resistance, R_t, and $\Delta R = R_t - R_I$, the decimal part of R_t. Then Equation 1 becomes

$$t = A - B\sqrt{S - \Delta R}$$

By assuming a linear 10-to-1 ratio of temperature to resistance, the approximate temperature, t_a, is

$$t_a = A - B\sqrt{S + 10\,\Delta R}$$

for the interval R_I to $R_I + 1$.

The quantity $A - B\sqrt{S}$ is the temperature corresponding to the integral resistance, R_I—that is, when $\Delta R = 0$. The difference, $t - t_a$, then provides the parabolic correction.

$$t - t_a = B(\sqrt{S} - \sqrt{S - \Delta R}) - 10\Delta R$$
$$= \left(\frac{B}{2\sqrt{S}} - 10\right)\Delta R + \frac{B}{8S^{3/2}}\overline{\Delta R^2} + \cdots$$

The next term of the series contributes less than 0.0005° C. up to a temperature above 1700° C., a temperature well beyond the practicable range of the instrument.

Calculations for the corrections are then readily performed by evaluating the coefficients of ΔR and $\overline{\Delta R^2}$ and then finding the correction for ΔR values of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0. Curves are then plotted with ΔR as abscissa and temperature correction as ordinate. There will be one such curve for each integral value of resistance, covering a range of about 10°.

For temperatures below zero, the same procedure is followed, except that before the parabolic-correction values are plotted, the values of the β-term are added. This process is straightforward, except for very low temperatures, where the value of the β-term correction to the temperature is enough to cause an appreciable shift in the value of the β-term itself. With β = 0.109, for example, this shift amounts to 0.0002° at −82° C. and 0.001° at −92° C.

Figure 1 shows a reduced set of parabolic-correction curves, covering the range from −92° to 218° C., for a specific resistance thermometer. For use, a chart covering this range would extend about 1 meter along the temperature axis and could be divided at the zero-correction line. These curves remain fairly insensitive to changes in the ice-point resistance of the thermometer. For such a shift corresponding to a temperature change of 0.010° C., the maximum change in correction-graph values, at $\Delta R = 1$, is of the order of 0.0005° C.

The following example illustrates the method of calculating the temperature corresponding to a given resistance. Assume the resistance to be 29.2764 ohms.

Temperature for $R_I = 29$ (from table)	34.544
10 times resistance decimal	2.764
Parabolic correction (from graph)	−0.015
Correct temperature	37.293° C.

Spot Test Detection of Antimony by Means of Gossypol

PHILIP W. WEST AND LOUIS J. CONRAD, *Louisiana State University, Baton Rouge, La.*

A NUMBER of spot tests for antimony have been reported in the literature (*4, 5, 7-10, 12*), but they often leave much to be desired, because of lack of specificity, poor sensitivity, instability or unavailability of the reagent, or a combination of these. The test which showed the most promise was based on the red color formed between trivalent antimony and 9-methyl-2,3,7-trihydroxy-6-fluorone. This test, as originally proposed by Wenger and Blancpain (*12*), lacked specificity, but the changes in procedure recently reported by Gillis, Hoste, and Claeys (*11*) have overcome this objection to some extent. Despite the improvements in the procedure several disadvantages remain: A number of ions still interfere, the reagent is not commercially available, the synthesis of the reagent is not satisfactory, and the solution of the reagent is not stable.

Boatner *et al.* (*2*) recently reported the use of antimony trichloride for the spectrophotometric determination of gossypol in cottonseed extract, based on the red color of chloroform solutions of the reaction product. The structure of the gossypol as reported by Adams and co-workers (*1*) contains two aromatic *o*-dihydroxy groupings. Proceeding from the gossypol-antimony trichloride spectrophotometric test and the aromatic *o*-dihydroxy group action displayed toward antimony by such compounds as pyrocatechol, pyrogallol (*7, 9*), and 9-methyl-2,3,7-trihydroxy-6-fluorone (*12*), an investigation was undertaken to study the use of gossypol as a spot test reagent for antimony.

REAGENTS

Gossypol Solution. Purified gossypol obtained from the Southern Regional Laboratory, New Orleans, La., was made up to a strength of 0.1% in reagent grade acetone.
Phosphoric Acid Solution. One volume of 85% reagent grade phosphoric acid was diluted with 4 volumes of distilled water.

EXPERIMENTAL

The spot plate as a medium for carrying out the test was found to be far superior to paper. The procedure followed in general was to adjust the antimony solution to between 0.4 N and 0.7 N with respect to hydrochloric acid and then add this solution to the spot plate, followed by 2 drops of gossypol solution per aqueous test drop. The evaluation of the test was made after 30 seconds had elapsed. Antimony in the range of 0.5 to 10 micrograms per drop gave an orange to red precipitate. Greater than 10 micrograms per drop gave a distinct red precipitate.

The determination of the limiting concentration and limit of identification was performed in accordance with the procedures described by Feigl (*6*). Interference studies followed in general the procedure discussed by West (*13*), except that the concentration of the antimony ion in solution was 0.01% and that of the ions used in the interference studies was 1%. Additional interference studies were made against a control having a concentration of antimony in solution of 0.002%. The interference studies were carried out on solutions acidified with hydrochloric acid in order to duplicate test conditions.

The ions investigated in the interference studies (Table I) are given below in their more common forms. It is realized that in many instances the ions concerned are present as complexes, but where structures of such complexes may be in doubt, only the valence of the central atom is indicated.

PROCEDURE

The solution to be tested must be acidic (0.4 N to 0.7 N with respect to hydrochloric acid) and should be gently warmed prior to making the spot test.
On a spot plate, 1 drop of the test solution is placed and to it is added 1 drop of the phosphoric acid solution, followed by 4 drops of the gossypol solution. An orange or red precipitate indicates the presence of antimony.
If the antimony in solution is in the quinquevalent state, it must be reduced to the trivalent form by means of sodium sulfite prior to carrying out the test.

REMARKS

The gossypol reaction, when used as a spot test, has a limit of identification of 0.5 microgram of antimony per drop of solution at a limiting concentration of 1 part in 100,000. No positive interferences were found when the interference tests were compared with controls containing 1 microgram of antimony per drop. Vanadate, dichromate, iodate, bromate, perosmic, and molybdate ions give negative or masking interferences. The interfering action of the oxidizing agents is due to the formation of quinquevalent antimony and/or the reaction of gossypol with the excess oxidizing agent. Molybdate, dichromate, vanadate, and perosmic ions react with gossypol alone to form colored precipitates: molybdate yellow, dichromate brown, and vanadate and perosmic green. Iodate and bromate ions do not form colors with the reagent alone, but in the presence of gossypol and antimony they cause the test color to be a faint green. Attempts to reduce these oxidizing agents in the presence of antimony in the spot plate were not satisfactory. The colors produced in these interfering reactions as well as the inherent colors of such ions as permanganate, chromous, and various platinum metal complexes tend to complicate the interpretations of test results.

The phosphoric acid used in the test prevents the interfering effects of ferric, quadrivalent titanium, stannous, stannic, and tungstate ions which would normally give the following colors with the reagent: ferric green, quadrivalent titanium and stannous orange, stannic red, and tungstate reddish brown. Zirconium, fluorides, thiosulfates, oxalates, and tartrates are masking interferences, but the interference due to fluoride can be removed successfully by the addition of boric acid which sequesters the fluoride as the tetrafluoroborate complex. Sulfide is not compatible with antimony. Nitrite ion oxidizes the trivalent antimony to the quinquevalent state which does not form a colored product with gossypol. The excess nitrite ion can be removed by warming the acidic solution and then the quinquevalent antimony can be reduced by the addition of sodium sulfite.

The acidity of the test should be controlled carefully. With hydrochloric acid concentrations below 0.4 N the antimony oxy-

Table I. Ions Investigated in Interference Studies

(Periodic table grouping)

I	II	III	IV	V	VI	VII	VIII	Miscellaneous
Li^+	Be^{++}	BO_2^-	CO_3^{--}	NH_4^+	S^{--}	F^-	Fe^{++}	CN^-
Na^+	Mg^{++}	$B_4O_7^{--}$	SiO_3^{--}	NO_2^-	$S_2O_3^{--}$	Cl^-	Fe^{+++}	$Fe(CN)_6^{---}$
K^+	Ca^{++}	Al^{+++}	Ti^{++++}	NO_3^-	SO_3^{--}	ClO_3^-	Co^{++}	$Fe(CN)_6^{----}$
Cu^{++}	Zn^{++}	Ga^{+++}	Zr^{++++}	$H_2PO_2^-$	SO_4^{--}	ClO_4^-	Ni^{++}	NCS^-
Rb^+	Sr^{++}	In^{+++}	Sn^{++}	HPO_3^{--}	Cr^{+++}	Mn^{++}	$RuCl_6^{--}$	
Ag^+	Cd^{++}	La^{+++}	Sn^{++++}	$P_4O_{13}^{------}$	$Cr_2O_7^{--}$	MnO_4^-	$RhCl_6^{---}$	
Cs^+	Ba^{++}	Tl^+	Ce^{+++}	$P_6O_{18}^{------}$	SeO_3^{--}	Br^-	$PdCl_4^{--}$	
$AuCl_4^-$	Hg^+		Pb^{++}	PO_3^-	SeO_4^{--}	BrO_3^-	OsO_5^{--}	
	Hg^{++}		Th^{++++}	HPO_4^{--}	MoO_4^{--}	I^-	$IrCl_6^{---}$	
				$P_2O_7^{----}$	TeO_3^{--}	IO_3^-	$PtCl_6^{--}$	
				VO_3^-	TeO_4^{--}	ReO_4^-		
				$HAsO_3^{--}$	WO_4^{--}			
				$HAsO_4^{--}$	UO_2^{++}			
				Sb^{+++++}	UO_4^{--}			
				Bi^{+++}				

chloride begins to precipitate, and above 0.7 N the color formation between the antimony and gossypol is suppressed.

Gossypol is reported to be unstable when stored at room temperature (3). In order to determine the effect of the decomposition of the gossypol on its use as a spot test reagent, the dry powder and an acetone solution of the material were stored in the dark at an average temperature of 80° F. At the end of 4 months the powder and the solution had darkened somewhat but both were satisfactory for use in the spot test.

CONCLUSION

Considering all the requirements for a good spot test, gossypol appears to be superior to any other reagent for antimony which has been previously reported in the literature. It is sensitive, highly selective, stable, and readily available. The spot test procedure is very simple, requiring no elaborate conditioning treatments or specialized techniques.

ACKNOWLEDGMENT

The authors wish to express their appreciation for financial

assistance given them under a contract with the Office of Naval Research.

LITERATURE CITED

(1) Adams, R., Geissmann, T. A., Dial, W. R., and Fitzpatrick, J. T., *J. Am. Chem. Soc.*, **63**, 2439 (1941).
(2) Boatner, C. H., Caravella, M., and Kyame, L., IND. ENG. CHEM., ANAL. ED., **16**, 566 (1944).
(3) Castillion, L. E., Hall, C. M., and Boatner, C. H., *J. Am. Oil Chemists' Soc.*, **25**, 233 (1948).
(4) Denigès, G., *Chem. Zentr.*, 1901, II, 1214.
(5) Eegriwe, E., *Z. anal. Chem.*, **70**, 400 (1927).
(6) Feigl, F., "Chemistry of Specific, Selective, and Sensitive Reactions," pp. 8–10, New York, Academic Press, 1949.
(7) Feigl, F., *Mikrochemie*, **1**, 74 (1923).
(8) Feigl, F., and Neuber, F., *Z. anal. Chem.*, **62**, 382 (1923).
(9) Feigl, F., and Ordelt, H., *Ibid.*, **64**, 41 (1924).
(10) Fresenius, C. R., *Ibid.*, **1**, 444 (1862).
(11) Gillis, J., Hoste, J., and Claeys, A., *Anal. Chim. Acta*, **1**, 291 (1947).
(12) Wenger, P., and Blancpain, C. R., *Helv. Chim. Acta*, **20**, 1427 (1937).
(13) West, P. W., *J. Chem. Education*, **18**, 528 (1941).

Introduction of Liquid Samples into the Mass Spectrometer

K. M. PURDY AND R. J. HARRIS, *Esso Laboratories, Esso Standard Oil Company, Baton Rouge, La.*

VARIOUS methods are now being used for the introduction of liquid samples into a mass spectrometer. The technique originally devised for this operation involves the sealing of a small sample of the liquid to be analyzed in a small, thin-walled glass vial, placing the glass vial in the sample introduction manifold, and rupturing the vial, thereby freeing the sample for admission through the leak to the ionization chamber. This method is very time-consuming and would be too cumbersome for a routine procedure.

The use of a sintered-glass disk covered with a pool of mercury as a valve to facilitate the introduction of liquid samples from a capillary pipet was first announced by the Atlantic Refining Company (2). A sintered-glass disk mercury valve is employed in a majority of the techniques now being used for the introduction of liquid samples into a mass spectrometer.

A new technique for this type of analysis (1) includes the use of a self-filling micropipet and a sintered glass–mercury valve. A principal disadvantage of this procedure is the introduction of air following every sample introduction, except when a mercury seal technique is employed, in which case accuracy is sacrificed.

A constant-volume capillary pipet was developed in the authors' laboratories to eliminate disadvantages of other methods of liquid sample introduction investigated. As shown in Figure 1, the pipet is so constructed as to be completely immersed in the
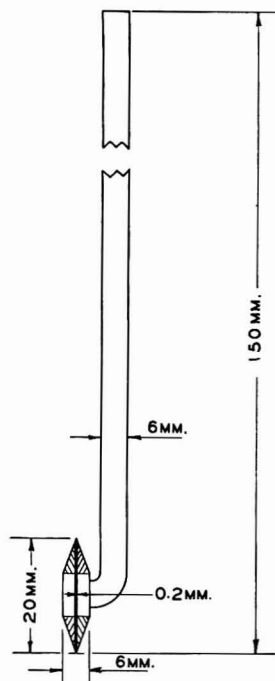

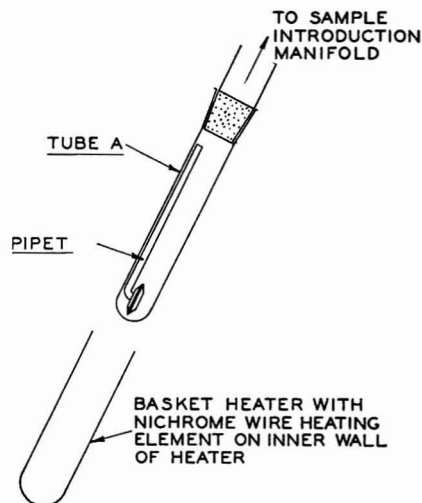**Figure 1. Constant-Volume Capillary Pipet**

Figure 2. Modified Apparatus for Viscous Samples

pool of mercury used in the sintered-glass valve, thus eliminating the introduction of air with every sample. A short length of capillary glass tubing is used in making this sampling device. It must, necessarily, be small because of pressure limitations in the mass spectrometer inlet system. The pipet shown in Figure 1 will deliver a volume of about 0.001 ml., if made according to the dimensions shown.

The bottom of the capillary tube is carefully ground to a point, so that positive contact with a single point of the fritted plate may be made. The top of the capillary is ground to a conical or rounded shape to prevent the collection of a small pool of liquid on top when the capillary is filled with sample. A glass rod of small diameter is secured to the middle of the pipet as a handle to facilitate handling the small pipet.

Emery paper has been found to be the most satisfactory medium for grinding these pipets. Emery paper No. 1 is used for

Sample	Date 1949	No. of Detns.	Average Peak Height	Standard Deviation	Probable Error
n-Heptane	5/18	7	254.8	0.89	0.60
n-Heptane	5/19	14	261.7	1.19	0.83
m-Xylene	5/20	7	1764.0	9.64	6.46
m-Xylene	5/31	10	1787.4	9.67	6.48

original grinding down, and this is followed by treatment with
No. 0 paper. A final polish is given to the ground surfaces with
No. 00 emery paper.

In sampling with the capillary pipet, it is essential that the
capillary be thoroughly clean. In order to fill the pipet, the tip
is merely touched to the surface of the liquid sample. The sample
is drawn into the pipet by capillary action.

A Corning Type F sintered-glass disk covered with mercury is
used to introduce the sample into the instrument. Enough mer-
cury must cover the fritted plate to ensure complete immersion of
the pipet when the tip contacts the surface of the fritted plate.
Reduced pressure beyond the fritted plate then pulls the sample
from the pipet and mercury enters the top of the pipet and re-
places the sample in the capillary. Extreme care must be exer-
cised in introducing samples with the pipet to make direct contact
with the fritted plate, avoiding any scraping or scratching of the
fritted plate surface, as small glass fragments easily plug the
capillary.

These pipets have been used satisfactorily in these laboratories
for analyzing liquids as high as C_{12} hydrocarbons with the inlet
system at room temperature. Typical reproducibility data are
shown in Table I.

Advantages of this procedure are that constant accurate vol-
umes are introduced for calibrations and analyses without a meas-
urement required, and no sensitivity calculations are required, be-
cause computations are made directly from peak heights.

For liquids too viscous to flow through the fritted plate, such as
C_8 alcohols, a modified method of sampling was developed, using
the apparatus shown in Figure 2. The pipet is filled with the
sample, the whole pipet is placed in tube A, and the tube is con-
nected to the instrument. Dry ice is packed around the tube
while it is evacuated. Then the heater is placed around the tube
and gradually heated until all the sample is vaporized from the
pipet.

LITERATURE CITED

(1) Friedel, A. R., Sharkey, A. G., and Humbert, C. R., ANAL.
        CHEM., 21, 1572–3 (1949).
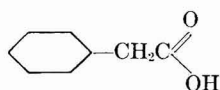(2) Taylor, R. C., and Young, W. S., IND. ENG. CHEM., ANAL. ED.,
        17, 811 (1945).

CRYSTALLOGRAPHIC DATA

37.   Phenylacetic Acid (α-Toluic Acid)

Contributed by DONALD G. GARBAR AND WALTER C. McCRONE
Armour Research Foundation, Illinois Institute of Technology, Chicago 16, Ill.

Structural Formula for Phenylacetic Acid

Phenylacetic acid is soluble in most common solvents. Crys-
tals for x-ray diffraction were grown from water solution and
those for optical studies by recrystallization on a microscope slide
from Cargille refractive index liquids.

CRYSTAL MORPHOLOGY
  Crystal System. Monoclinic.
  Form and Habit. Tablets flattened parallel to and lying on
100 showing orthopinacoid {100} and clinodome {011}. Occa-
sionally the clinopinacoid {010} is found.
    Axial Ratio. $a:b:c = 2.88:1:2.04.\ \ 2.90:1:2.06$ (1).
    Interfacial Angles (Polar). 011 ∧ 0$\bar{1}$1 = 53° − 12′.
    Beta Angle. 102°; 101° (1).

X-RAY DIFFRACTION DATA
  Space Group. C_{2h}^5 (1).
  Cell Dimensions. $a = 14.36$ A.; $b = 4.98$ A.; $c = 10.17$ A.
$a = 14.2$ A.; $b = 4.90$ A.; $c = 10.0$ A. (1).
  Formula Weight per Cell. 4.
  Formula Weight. 136.14.
  Density. 1.228; 1.262 (x-ray).

Principal Lines

d	I/I_1	d	I/I_1
14.38	1.00	3.16	0.16
7.08	Very weak	3.02	0.13
6.32	Very weak	2.89	Very weak
5.91	Very weak	2.79	Very weak
5.05	0.11	2.69	0.15
4.74	0.16	2.55	Very weak
4.49	0.53	2.48	Very weak
4.33	0.77	2.41	Very weak
4.19	Very weak	2.32	Very weak
4.08	0.22	2.26	Very weak
3.81	0.14	2.03	Very weak
3.66	Very weak	1.99	Very weak
3.52	0.59	1.87	Very weak
3.34	0.16	1.78	Very weak
		1.61	Very weak

OPTICAL PROPERTIES
  Refractive Indexes (5893 A.; 25° C.). $\alpha = 1.558 \pm 0.001$;
$\beta = 1.569 \pm 0.002$; $\gamma = 1.671 \pm 0.005$.
  Optic Axial Angles (5893 A.; 25° C.). $2V = 39°$; $2E = 67°$.
  Dispersion. $v > r$ slight.
  Optic Axial Plane. 010.
  Sign of Double Refraction. Positive.
  Acute Bisectrix. γ.
  Extinction. $\beta \wedge c = 5°$ in obtuse β.
  Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.599$;
R (calcd.) = 35.7; R (obsd.) = 37.9.
  FUSION DATA. Phenylacetic acid melts without decomposition
at 76° C. It crystallizes without seeding, forming flattened rods
lying on 100 and some crystals elongated parallel to c lying on
010. The crystal front in a mixed fusion usually has ill-defined
profile angles due to solubility. However, a Canada balsam mixed

Figure 1.   Phenylacetic Acid

Left.   Crystals grown from solution on microscope slide
Right.   Crystals grown from melt, crossed Nicols

fusion will permit measurement of the β angle and extinction before the crystals become too rounded. Some of the crystals from fusion show a slightly inclined Bx_a figure with $2V = 39°$, $v > r$, ($+$). No evidence of polymorphism was observed.
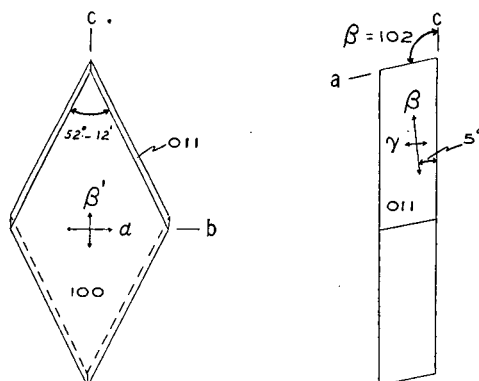

Figure 2. Orthographic Projection of Typical Crystal of Phenylacetic Acid

It is a pleasure to acknowledge the help of Ann Humphreys in determining the powder x-ray diffraction spacings and intensities.

LITERATURE CITED

(1) Patterson, A. L., *Phil. Mag.*, 3, 1252 (1927).

CONTRIBUTION. 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.

Semimicro Qualitative Analysis. *William C. Oelke.* ix + 377 pages. D. C. Heath and Co., 285 Columbus Ave., Boston 16, Mass., 1950. Price, $3.75.

In the reviewer's opinion, this book will go a long way toward satisfying the increasing desire of teachers of qualitative analysis for a text which assigns to the subject its appropriate importance in the chemistry curriculum. Oelke's attempt to emphasize the laws of chemical equilibrium as the logical basis for the analytical procedures utilized is highly successful; the correlation between theory and practice leaves little to be desired.

The discussion of the theory of electrolytic solutions and their equilibria is in modern terms and will serve as an excellent background for students who go on to advanced courses in chemistry. The section on acids and bases is particularly well written.

The descriptive material dealing with the analytical scheme is clear and concise. In addition to the common inorganic tests for the various ions, tests with organic drop reagents are frequently given. An intelligent preliminary discussion prepares the student for the use of these reagents. An important factor contributing to the value of the descriptive portion of the text is the utilization of current knowledge and concepts concerning the chemical nature of the various ions and precipitates encountered in the analytical scheme. The use of appropriate literature references to document the material and to serve as stimuli to the more curious students is warmly commended.

Oelke has carefully selected the wide variety of exercises and problems found at the end of each chapter; they well serve to illustrate and illuminate the principles of both the theory and practice of qualitative analysis.

Although the book is primarily designed for a sophomore course, there are abbreviated cation and anion analytical schemes

which may be employed by those wishing to use the text for students in the latter part of the freshman year. The book is highly recommended for the serious consideration of teachers of analytical chemistry.   JACOB KLEINBERG

Philadelphia Section Meeting

The Chemical Education Committee of the Philadelphia Section, AMERICAN CHEMICAL SOCIETY, will present a continuation course on "Advances in Chemical Analysis," at the Philadelphia College of Pharmacy and Science on Tuesday evenings. Information is available from R. E. Vener, Drexel Institute of Technology, Philadelphia 4, Pa.

October   17   Ion Exchange in Analysis. W. RIEMAN III, Rutgers University
October   24   Countercurrent Distribution. L. C. CRAIG, Rockefeller Institute for Medical Research
October   31   Isotope and Radioactive Tracer Techniques. G. G. MANOV, Atomic Energy Commission
November 7   Determination of Organic Functional Groups by Chemical Means. S. SIGGIA, General Aniline and Film Corp.
November 14   Flame Photometry. T. E. WEICHSELBAUM, Washington University, St. Louis
November 21   Polarography of Organic Compounds. P. J. ELVING, Pennsylvania State College
November 28   X-Ray Absorption. H. A. LIEBHAFSKY, General Electric Co.
December 5   Chromatographic Separations. A. L. LEROSEN, Louisiana State University
December 12   Statistical Evaluation of Analytical Data. W. J. YOUDEN, National Bureau of Standards

This continuation course conflicts with the meetings planned by the Analytical and Microchemical Group for November and December as announced here last month. The meetings will be held at the Harrison Laboratory of Chemistry, University of Pennsylvania, 34th and Spruce St., Philadelphia.

Chromatography of Colorless Organic Compounds. JOHN W. SEASE, Wesleyan University. November 2
Determination of Molecular Weight. ROBERT E. KITSON, Polychemicals Department, E. I. du Pont de Nemours & Co., Inc. December 7

Indiana Symposium on Analytical Chemistry

The Indiana Section of the AMERICAN CHEMICAL SOCIETY has announced its fourth Symposium on Analytical Chemistry, to be held at the Indiana World War Memorial, Indianapolis, November 4 from 8 A.M. to 5 P.M. The program includes: Organic Analytical Reagents. FRANK WELCHER. Radioactive Assay. GEOFFREY T. GLEASON AND D. L. TABERN. Anhydrous Titration. JOHN RIDDICK. Paper Chromatography. WALTER WINSTON. Analytical Spectroscopy. RICHARD C. LORD.

Optical Society of America. Cleveland, Ohio, October 26 to 28
Fourth Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 29 to February 1, 1951
Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. William Penn Hotel, Pittsburgh, Pa., March 5 to 7, 1951
Fourth Annual Summer Symposium. Washington, D. C., June 14 to 16, 1951

Apparatus for Packing Columns with Single-Turn Helices.
O. C. W. Allenby and C. L'Heureux, Central Research Laboratory, Canadian Industries Limited, McMasterville, Quebec, Canada.

FILLING a distillation column by hand with single-turn helices is a tedious and time-consuming operation. For greatest column efficiency, the helices must be separated from one another during the packing process and as far as possible introduced singly. To pack even a small laboratory column properly requires a number of hours.

A simple apparatus which was designed to overcome this difficulty has been in use for the past 3 or 4 years in this laboratory. The helices are separated by a jet of air which, by whirling them in a round-bottomed flask, untangles them from their neighbors. Single helices then are carried by the air stream up through the neck of the flask and are delivered to the top of the column by a glass tube.
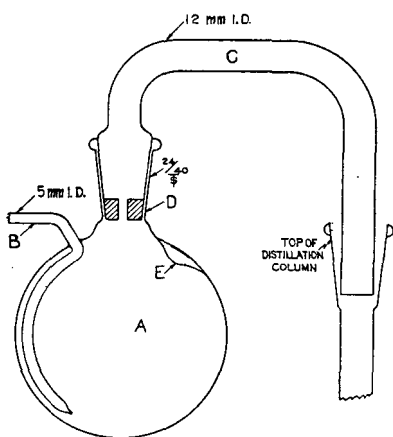

Figure 1

The apparatus consists of a round-bottomed flask, A, of 500-ml. capacity fitted with a 24/40 standard-taper joint (see Figure 1). A side arm, B, is blown into the side of the flask and extends downward, following the contours of the inside until it reaches almost to the bottom. The end of the tube is flattened to give a broad jet approximately 1 × 7 mm. in inside dimensions. A piece of glass tubing, C, connects the flask through a 24/40 joint to the head of the distillation column. The end of the joint is partially closed by a short section of rubber stopper in which a hole 5 mm. in diameter has been bored, D.

In operation, approximately 25 grams of helices are introduced into the flask and the side arm is connected to a source of compressed air. Only a comparatively low pressure is required, several pounds per square inch being sufficient. Suitable precautions should be taken if a source of air under high compression is used because of the possibility of some blockage occurring in the system with subsequent rupturing of the flask. The air flow is adjusted so that the helices are whirled in a circular motion, thus gradually loosening up agglomerates and producing single isolated helices. If the flow of air is correct, these single helices are carried up with the air current and over into the column. In order to prevent the whirling mass of helices from striking the far lip of the neck of the flask, two elongated depressions are made in the flask just below the neck each at right angles to and on either side of the center of their line of travel (E, Figure 2). This serves to deflect the major portion of the helices slightly downward. Of those which pass through the gap, the single helices are carried by the air stream up through the neck and into the column. When the quantity of helices remaining in the flask has been reduced by about one half, refilling to the original amount maintains a smooth circulation. Difficulty may occasionally be encountered on very dry days with static electricity built up as a result of the circulation of the helices in the flask. This can be overcome by moistening the air by passing it through a scrubber filled with water before introduction into the apparatus.

With the above equipment, a 1.5 × 52 cm. column was packed with single-turn $^1/_8$-inch glass helices in 20 minutes. A total o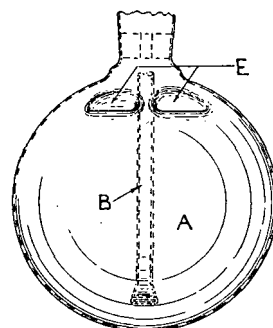f 34.2 grams of helices was introduced into the column and with a carbon tetrachloride-benzene mixture at total reflux a plate value of 9 was obtained. To pack the same column by hand required 4 hours of concentrated effort, 33.2 grams of helices were introduced, and the plate value under the same operating conditions was also 9.

The equipment has also been found to operate satisfactorily with 32- to 34-gage $^1/_{16}$-inch nickel helices.

Figure 2

Source of Error in Kjeldahl Microdeterminations. W. J. Wingo, O. L. Davis, and Lee Anderson, M. D. Anderson Hospital for Cancer Research, Houston, Tex.

IN nitrogen determinations by the Kjeldahl micromethod, it is a common practice to boil the distillate before titration in order to sharpen the end point. Frequently, the water is drained from the condenser for a minute or more at the end of the titration in order to "steam out" the condenser. Both practices become sources of error when boric acid, instead of a strong acid, is used to trap the ammonia distilled from the sample.

Recently, in a series of Kjeldahl microdeterminations by the method of Miller and Houghton [J. Biol. Chem., 159, 373 (1945)], the distillates were boiled before titration. The boiling sharpened the end point, but the results were invariably low. A search of the literature showed that Markly and Hann [J. Assoc. Offic. Agr. Chemists, 8, 455 (1925)] had studied the loss of ammonia from heated ammonium borate solutions. They found that after heating for 30 minutes there was no loss at temperatures below 50° C., but there was an increasing loss at higher temperatures; at 100° only 34.75% of the ammonia remained.

Even bringing the distillate to the boiling point entailed a small loss of nitrogen, which increased with the time of boiling; 5 minutes' boiling gave losses of about 7%. Titration of the distillate without any heating gave results of satisfactory accuracy; recoveries of 99.5 to 100.3% were obtained on solutions of ammonium sulfate and on a series of amino acids.

Because some schemes for Kjeldahl microdeterminations involve a final steaming out of the condenser, this technique was also studied by steaming out the condenser at the end of a distillation routine which was known to give quantitative results. Low recoveries were obtained whenever the distillate was significantly heated by the steam.

The dangers to accuracy inherent in heating Kjeldahl distillates after trapping the ammonia with boric acid have been known for many years, but recent reports on the use of this method have not mentioned this source of error.