ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editor

The Lisbon Analytical Congress

WORD has now come to us through official channels that an international analytical congress will be held in Lisbon, Portugal, in September of next year, under the auspices of the Analytical Section of the International Union of Pure and Applied Chemistry.

We congratulate our Portuguese associates and pledge them and the IUPAC analytical section our active support in order to help make the 1956 gathering in Lisbon an outstanding success in every way, comparable to the memorable Oxford meeting of a few years ago.

The staging of another international analytical congress demonstrates the full vigor of the Analytical Section of IUPAC, and American analytical chemists can be very proud of the leadership given it by I. M. Kolthoff as president, and S. E. Q. Ashley as secretary. Both have traveled to Europe extensively on IUPAC matters in recent months. Their intense interest in developing a closer relationship scientifically and personally with the analysts of other countries constitutes a splendid example for all of us to follow. It is upon such a firm basis that international good will and understanding are built.

The Division of Chemistry and Chemical Technology of the National Research Council (not the AMERICAN CHEMICAL SOCIETY) is the U. S. official connection with IUPAC. The division, under the chairmanship of William J. Sparks, has endorsed most enthusiastically the Lisbon analytical congress and is maintaining close liaison with the officers of the section and with Professor D. Antonio Pereira Forjaz, president of the organizing committee. Physical arrangements for the congress are being handled by Professor Pierre Laurent of the Instituto Superior Tecnico, Lisbon. The fact that IUPAC is staging a biennial meeting in Zurich in July of this year will mean that many analytical chemists will be there to help in planning for the Lisbon analytical congress in 1956.

It is the general feeling of the officers of the IUPAC Analytical Section and the Division of Chemistry and Chemical Technology of the National Research Council, that papers offered for presentation at the Lisbon meeting by American analysts should be screened by a board of referees appointed by the Division of Chemistry and Chemical Technology. We believe this to be a very practical, a very excellent idea.

ANALYTICAL CHEMISTRY would seem to be the logical choice for the ultimate publication of papers presented by Americans at the Lisbon congress. The editors will feel highly honored if this invitation is accepted. All authors should be aware that manuscripts offered for publication in the journal, regardless of the place or occasion of presentation, are subjected to our usual rigorous reviewing. The editors will, however, if this journal is selected as the medium for publication of the papers presented by Americans, publish them together in a single issue and with appropriate special editorial treatment.

More specific and detailed information on the Lisbon analytical congress will appear in subsequent issues of this journal and in *Chemical and Engineering News*.

In writing about the congress we are reminded of the fact that analysts are in for a very busy year in 1956. Running through the list very hurriedly, we can think of the Pittsburgh Analytical Conference, the Louisiana State University Symposium, the spring and fall national meetings of the ACS, the analytical session of the Gordon Research Conferences, and in 1956 a very special Summer Analytical Symposium—the annual event cosponsored by the Division of Analytical Chemistry and ANALYTICAL CHEMISTRY.

For the first time in the history of the summer analytical symposia, one will be held in the Far West—on the campus of the University of California at Los Angeles. Extended to a three-day meeting, there will be three sections: (A) Rapid Methods of Analysis, (B) Analysis of Industrial Wastes, and (C) Analytical Problems Encountered in Biological Systems. More about this special affair later.

The year 1956 will be a particularly strenuous one, but we look upon this increased activity simply as an indication of the ever-increasing importance of the broad field of analysis. A decade or so ago no one supposedly in his right mind would have pictured the analysts as the modern gypsies of the 20th century or, perhaps, we should have said the modern Gullivers. It is a lot of fun, of course, but of more serious importance it means that our special field of endeavor is progressing scientifically at a phenomenal rate of speed and meetings are essential for the fast and accurate dissemination of new knowledge upon which new industries are being built and old ones rejuvenated.

Operating a Petroleum-Research Analytical Laboratory

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Analytical expenses represent a substantial part of petroleum research costs; therefore, all analytical laboratory operations should be examined carefully. Frequently, laboratories which would not consider using inferior testing methods or equipment depend upon accounting and communication procedures that are not consistent with good office practice. Any system of handling samples, keeping records, and preparing reports depends upon the philosophy of the organization using it. This paper describes some of the procedures used by the research analytical group of the Union Oil Co. of California and the general policies that influenced their development. Sample handling from the time a client fills a sample bottle until the sample is returned or discarded is discussed and the advantages of printed forms and special equipment are described. The preparation and application of weekly and monthly reports for the aid of supervisory personnel in the conduct of laboratory operations are elucidated.

THE criterion of the efficiency of an analytical laboratory to those seeking its services is the accuracy of the result and the speed with which it is obtained; the measure used by the analyst is the degree to which his technical training is utilized; and in addition, research management is concerned with whether the services needed are supplied at reasonable cost. It is the function of analytical supervision to reconcile these somewhat divergent viewpoints and to provide services that as nearly as possible satisfy everyone. To accomplish this, considerable information must be collected, correlated, and made available where and when needed, and all operational procedures in the laboratory must be well coordinated.

The information needed by the one submitting the request, hereinafter designated as the client, is how to submit a sample and request sheet and how the answer will be returned. Analytical personnel need to know the accuracy required, approximate composition, contaminating substances, possible hazards, priority of work, whether or not the analysis is a specification test, and, in general, as much background as possible on the sample source and the use to which the data are to be applied. Supervisory personnel, from the lowest level in the analytical laboratory to the head of the department, must know how well the services are being provided, whether the laboratory is being operated efficiently, what the work is costing, and the present and anticipated work load. The basic information for these purposes is procured from specially designed work request forms and monthly work load estimates submitted by the client and from time sheets submitted by analytical personnel.

The operational procedures that must be coordinated are the handling of work requests and samples, the recording of analytical data, the selection of the proper method for unusual samples, and the preparation of statistical reports. The handling of requests and samples is expedited by mechanical duplication of request forms and by the use of special tags and storage facilities. The recording of analytical data is simplified by printed work sheets. A centralized punched-card file system makes possible the rapid selection of the proper method. The preparation of statistical reports for supervision is aided by the use of punched cards and IBM equipment.

This paper is restricted to describing the operational techniques that the analytical group of the research and process department of the Union Oil Co. of California has found effective. The techniques described are the result of a study of the procedures of similar laboratories in the industry and several years' experimentation in this laboratory. Several interesting papers on laboratory operations have appeared recently in the literature (*1–5*). The analytical group of 65 persons located at the Brea Research Center is composed of three sections which are subdivided into eleven laboratory units. This group provides the complete line of testing required by petroleum research investigations and, in addition, furnishes consulting services and performs analytical research. During 1953 some 42,000 samples were processed, 29,000 by the chemical section and 13,000 by the other two sections. The number of tests performed on chemical section samples ranged from 1 to 35 and averaged about 2. The samples analyzed by the instrumental sections were counted as one test per sample per instrument regardless of the number of components reported.

This analytical group has developed an organization that facilitates coordination of operations and distribution of supervisory responsibilities to the analytical chemists. This group is composed of a supervisor, a secretary, two clerks, 22 chemists, and 39 technicians. All of the chemists in the group have at least a bachelor's degree or its equivalent and many of the technicians have had college training in science.

Reporting to the supervisor are three administrative section leaders and two coordinators. The coordinators assist in bringing the operations of the group together into a harmonious relationship: one to coordinate sample handling, records, and statistical reports; and one to develop and maintain a readily accessible up-to-date file of analytical methods. Reporting to the section leaders are unit leaders who are charged with direct supervision of laboratory work and personnel.

The procedures used in any laboratory and the facilities provided will be influenced by the philosophy, policies, and rules of the organization of which it is a part. To aid in understanding why some of the techniques described were adopted, the pertinent policies that guide the analytical group are enumerated.

1. Research investigations must not be delayed because of slowness in obtaining analytical services.
2. Analytical work shall be centralized unless definite savings in analysis time dictates otherwise.
3. Analytical time shall be charged to the project or department making the request in order to obtain proper distribution of labor costs and thus to ensure accurate data on research expenditures.
4. The analytical group places no restrictions on the amount of routine analytical service that may be requested by the professional men at the Brea Research Center. Control is maintained by the supervisors of the clients in accordance with their budgets.
5. All regular samples should be completed within 5 working days, complaint samples within 3 working days, and rush samples within 1 day.
6. Requests for rush work are discouraged because granting priority to one client usually has an adverse effect on the services that may be rendered to others. Rush samples must be authorized by a supervisor before acceptance for special service. The analytical group keeps complete records on the influx of priority work and watches its influence on the operation of the laboratory. If the situation begins to get out of control, the supervisors are warned that requests of this type must be curtailed if the value of priorities is to be preserved.
7. Analytical research problems are assigned to the analytical chemists in the group who have the best background for their solution. There is no section for analytical research.
8. Analysts (chemists and technicians) are assigned specific tests—for example, infrared absorption analyses or lamp sulfurs.

Assignments are rotated periodically to maintain flexibility and minimize monotony.

9. All paper work and movement of samples is done by clerks, and all routine and semiroutine tests are performed by technicians, if practical.

10. Time-saving equipment and special stationery are used to conserve manpower.

COORDINATION OF OPERATIONS

Most requests for analytical work are received from the research and process department and are for the analysis of petroleum products by generally accepted procedures. To help ensure that this work is done rapidly and efficiently and to the satisfaction of clients, special tags and request forms are used. All tags and forms are described in detail in the section on expediting facilities.

When submitting samples for analysis, a client must attach appropriate identification and handling tags to all containers and fill out a work request form. Any reasonable system of numbering or identifying samples is acceptable.

Twice daily a delivery man visits all laboratories at the research center and brings work requests and samples to the analytical sample receiving office. Clients are encouraged to use this delivery service because the research center is spread over 22 acres; however, when samples require rush handling or discussion of a special analysis, they may be brought by the clients.

The sample-receiving clerk checks all incoming requests to see that they are complete, correct, and legible, and that sample identifications on these requests agree with those on the sample tags. Forms which are incorrect are marked and returned to the client.

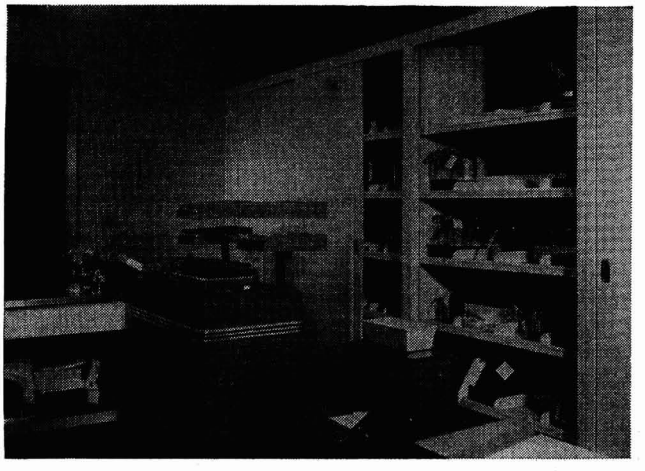


Figure 1. Sample storage compartments and Thermo-Fax duplicator

The receiving clerk checks the samples to see that they are properly tagged and that sufficient material has been submitted to permit all the tests requested. After samples are checked, those requiring analysis by the emission spectrograph, electron microscope, or x-ray equipment are sent directly to the laboratory concerned. Generally, clients supply separate samples for these units. Other samples are placed in compartments in the walls of the receiving office, as shown in Figure 1. The clerk assigns space by attaching compartment tags to sample containers. These tags, prenumbered to correspond to compartments, are selected at random for regular samples, and are specially marked when samples must be stored in refrigerators or baths. The space assignments are recorded on the request forms. Numbered squares are circled on the tags to indicate how many tests are to be preformed on the contents of each container.

After sample locations have been recorded, the clerk makes sure that one copy of the request, in addition to the original, is available for each test desired. If more copies are needed, they are made on a Thermo-Fax duplicator. A single test is checked on each copy and the copies with routine tests checked are placed in pigeonholes by test type. Copies for nonroutine tests are sent to unit or section leaders for assignment; the original copy is given to the card clerk.

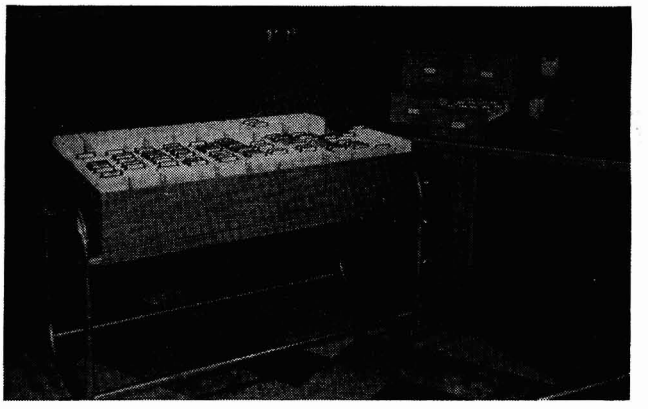


Figure 2. Test card bin

Upon receiving a request from the sample clerk, the card clerk prepares a deck of cards for each sample listed on this request by selecting appropriate cards for each test from a bin containing prenotched and premarked cards for the most commonly used tests. This bin is shown in Figure 2. The clerk writes the sample number on each card and notches the deck with a groover, according to client, project number, and priority. The cards are placed in an active file by test, and each day that they remain in this file, up to a total of 10 days, a notch is made in the service portion to indicate the time the sample has been in the laboratory.

Analysts obtain requests from the pigeonholes. When ready to start work, they find their samples in the compartments indicated on the requests. After the portion of material needed for the specific test has been withdrawn, the analyst initials the lowest unmarked square on the compartment tag and returns the container to the shelf at once, so that the sample will be available for other analysts. If the analyst initials the square circled by the sample clerk, he knows that his is the last test, and the color of the sample tag indicates whether the material should be discarded or placed on a separate shelf for return to the client by the delivery service. Compartment tags from completed samples are returned to the sample clerk, who makes a new tag carrying the same number so that the compartment may be reassigned.

Analysts record all data, calculations, and results on printed work sheets, keeping a carbon copy if they wish. Unit leaders check work sheets daily for completeness of data and possible errors and send them to the card clerk who uses them to keep the punched cards up to date. They are finally filed chronologically by test in the permanent analytical file.

The analyst records results on his copy of the request form, and, after approval by the unit leader, the copy is returned to the client by the delivery service. Results are reported as soon as they are available rather than being held until all tests requested by the client on a particular sample have been completed; also, because the request form is used as a report sheet, analysts do a minimum amount of writing and transcription errors are avoided. A flow diagram showing the routing of work requests and samples is shown in Figure 3.

All analysts keep time sheets on which they record the number of hours they spend working on various projects. In addition to indicating the project numbers, they also classify each labor charge according to unit number and labor type as described in the paragraph on time sheets. At the end of each pay period,

these time sheets are processed by the accounting department with IBM equipment. The tabulated information obtained is used for payroll purposes, to distribute analytical labor charges to project accounts, and to prepare monthly operational reports for the analytical group.

Requests requiring analytical research such as the evaluation and comparison of various methods, improvement of existing procedures, or the development of new techniques may originate anywhere within the company and, after approval by a responsible authority, are sent to the supervisor of the analytical group. Special or unusual analyses are requested in a similar manner. To expedite and correlate handling, requests are sent to a committee composed of the three section leaders and the two coordinators of the analytical group. This committee, known as the Committee on Analytical Research and Development, has the following duties:

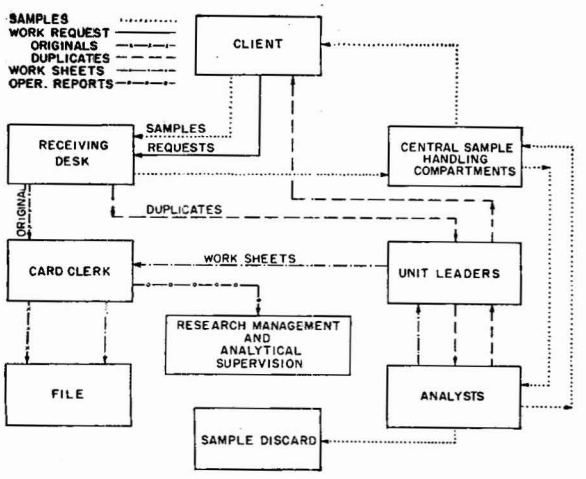


Figure 3. Routing of work requests, samples, and reports

1. Consider the technical soundness of each request, decide upon the most likely approach to the problem, and assign it to the appropriate section within the group.
2. Consider the work load and decide if the job can be completed within a reasonable time with present personnel. If not, notify the client that he must discuss the priority of the work with the analytical supervisor.
3. Notify the client of the estimated cost of the work, the estimated completion date, and to whom the project was assigned.
4. Watch that work on the research projects does not lag and provide technical advice when needed.
5. Compile a monthly report summarizing the current status of each assigned project.

The committee does not attempt to evaluate the relative importance or justification of clients' requests for research and development work.

The research request is reproduced on the Thermo-Fax duplicator so that all members of the Committee on Analytical Research and Development and the analytical chemist to whom the project is assigned may have a complete description of the assignment. The project is not considered completed until a report has been written, cleared through the committee, and sent to the supervisor for distribution or transmission to the client.

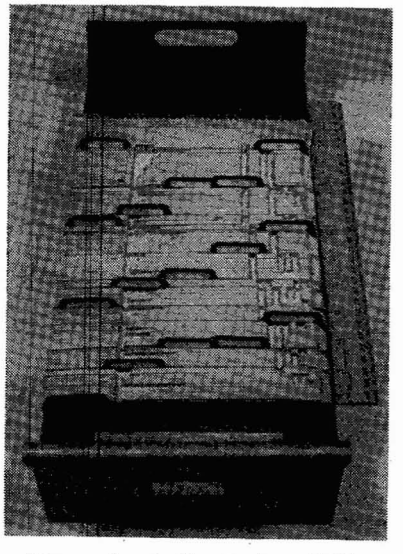


Figure 4. Active test card file

DEPARTMENT	PROJECT GROUP	CHEMICAL			
		HYDROCARBON UNIT		ELECTROMETRIC UNIT	
		TESTS COMPLETED	HOURS CHARGED	TESTS COMPLETED	HOURS CHARGED
RESEARCH & PROCESS	GASOLINE FUEL LUBE OIL GREASE CATALYSIS CHEMICALS PRODUCTION ANALYTICAL PILOT PLANT MISC.				
MANUFACTURING					
TRANSPORTATION					
MARKETING					
	TOTAL				
PRIORITY TESTS COMPLETED	RUSH				
	COMPLAINT				
PERCENTAGE OF TESTS COMPLETED WITHIN FIVE DAYS					
UNCOMPLETED TESTS	BEGINING OF MONTH				
	END OF MONTH				
	CHANGE				

Figure 5. Portion of monthly report on completed tests, labor, and work load

Frequently requests are received from outside the department for analytical services that do not involve research. They are also processed by the Committee on Analytical Research and Development, and the completed work is reported by letter to the client. A weekly reminder is prepared for the supervisor and section leaders listing by title the assignments that have not been completed.

In addition to the progress reports listed, periodic statistical reports are prepared to assist in the administration of the analytical group.

REPORTS

The active punched-card file is used for daily control and to prepare reports on the work load of the analytical group. Only a quick glance at the file is needed to obtain a good approximation of the total number of tests which have been requested but not completed. A closer examination of the service section of the cards reveals how long the tests have been in the laboratory and thus serves as a warning to the unit leaders when work on any test lags. Figure 4 illustrates this application of the file. This visual check is possible because a card is made for each test and the number of cards is indicated by the thickness of the file. The number of tests can be estimated with an accuracy of approximately 1% by measurement with a ruler. Although cards are normally filed according to test, it is a simple matter with the sorting needle to arrange them by client, project number, priority, service, age, or any combination of these factors that is desired.

A weekly tabulation of data obtained from this file is distributed to the unit leaders, section leaders, and the supervisor. This report gives, by laboratory units, data on uncompleted work and service age. Ordinarily when a test is not completed within 10 days, something is wrong and an investigation by the unit leader must be made. Section leaders and the supervisor use the information presented in this report to help determine what shifts in personnel may be necessary to balance the work load. Only about an hour of the card clerk's time is required to prepare this report.

Monthly, the analytical group receives estimates of the routine work expected for the ensuing 2 months from the other groups in the department. These are compiled by the coordinator of samples, and information on previous months is added for the

use of analytical supervisory personnel in determining trends in work load, in anticipating personnel requirements and distribution, and in planning for new tests not ordinarily performed by the laboratory.

Each month a 7-page operational report is prepared for supervisory personnel of the analytical group. The first 2 pages contain an organization chart and general information on the status of personnel. The third page contains a table of instrumental operability. Data for this table are obtained from downtime cards located at all major pieces of equipment in the laboratory. The operators of these instruments record the number of hours the equipment is inoperative each month because of malfunctioning, shutdowns for preventive maintenance, or modifications. A written explanation is given for all operabilities of less than 90%. The final pages contain information on the number of tests completed during the month shown as a function of the analytical laboratory unit and submitting group, service in terms of the percentage of tests completed within 5 days, and tests uncompleted on the last day of the month. In addition, information is presented giving labor distribution by type, by project, and by laboratory unit. Information presented on the final pages is obtained from test cards and IBM tabulations of time sheet data. The value of these tabulations to the analytical group supervisory personnel may be better realized by reference to Figures 5 and 6.

UNIT / TYPE	CHEMICAL	
	HYDROCARBON UNIT HOURS	ELECTROMETRIC UNIT HOURS
SUPERVISION AND TRAINING		
SAMPLE HANDLING AND RECORDS		
CALIBRATION AND MAINTENANCE		
SAMPLE ANALYSIS		
ANALYTICAL RESEARCH		
COOPERATIVE WORK		
VACATION AND HOLIDAY		
SICK LEAVE		
TOTAL		

Figure 6. Portion of monthly report on labor by type and unit

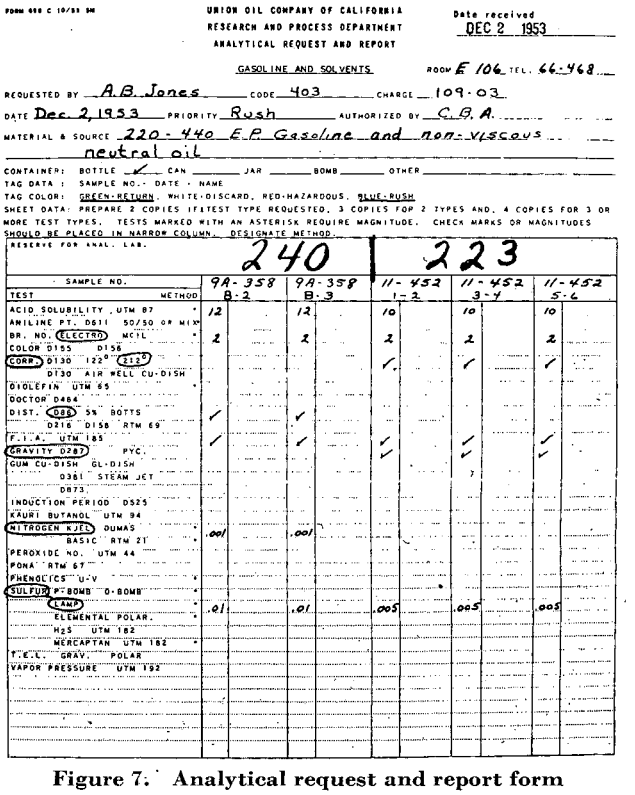
All labor data are processed by IBM equipment and need only be transcribed to be included in the operational report. The compilation of the entire monthly report requires about 24 hours of clerical time.

The application of these reports in coordinating and following the activities of an analytical group will be obvious to those having administrative duties in such groups. The work load report, for example, is used by unit and section leaders at weekly meetings to redistribute personnel so as to prevent impaired service from occurring in any test area. These restrictions may start because of sickness or vacation of the analyst normally assigned to that test, equipment difficulties, or a suddenly increased influx of samples. This report, in conjunction with daily inspections of the active test card file, gives unit and section leaders a complete picture of the routine work load not only of their unit but also of the entire group. The current work load report together with the anticipated work load report previously described provides a warning of where and when overloads may occur. Unfavorable situations which cannot be remedied by personnel shifts are avoided either by the voluntary reduction in demand for analytical services by clients upon being notified of impending trouble or by an increase in analytical group personnel. The anticipated work load report, in addition to indicating what shifts in personnel are needed, provides information on equipment needs, when shift work must be used, and permits the training of analysts and the installing of equipment for new or unusual tests before routine samples are received.

The monthly operational report is primarily for higher levels of supervision and for research management. The principal uses of this report are in determining trends in the magnitude and type of the work load and thus to indicate long-range requirements for space, equipment, and personnel. This information is especially useful, as it is available in detail for the individual laboratory units. The over-all efficiency of the group—that is, the efficiency that nearly as possible satisfies the client, the analyst, and management—may be derived from a study of this report. Furthermore, its value to the analytical group and to the other groups in the department in the preparation of budgets should be apparent.

EXPEDITING FACILITIES

Request-Report Forms. To simplify requesting and reporting of regular analytical service, printed forms are used that serve both for submitting samples and for reporting results. The printed forms make it convenient for the client to indicate the tests desired with minimum writing and also serve as a reminder of information needed for effective analysis of the sample. The client may list several samples for the same project on each sheet and may use any system of identifying the sample desired. The advantages of the printed checkoff form, in saving the client's time, and at the same time providing the analyst with complete, specific, and legible information, outweigh the disadvantage that some clients may check more tests than are really needed. The following forms, grouping tests that apply to one type of sample on a single sheet, are currently in use: inorganic analysis; light oils; heavy oils; grease; wax and asphalt; emission spectrograph (quantitative); emission spectrograph (qualitative); infrared and ultraviolet spectrophotometers; mass spectrometer; and special (no test listed).



UNION OIL COMPANY OF CALIFORNIA
RESEARCH AND PROCESS DEPARTMENT
ANALYTICAL REQUEST AND REPORT

Date received DEC 2 1953

GASOLINE AND SOLVENTS ROOM E 106 TEL. 66-468

REQUESTED BY A.B. Jones CODE 403 CHARGE 109-03

DATE Dec. 2, 1953 PRIORITY Rush AUTHORIZED BY C.B.A.

MATERIAL & SOURCE 220-440 E.P. Gasoline and non-viscous neutral oil

CONTAINER: BOTTLE ✓ CAN ____ JAR ____ BOMB ____ OTHER ____

TAG DATA : SAMPLE NO.- DATE - NAME

TAG COLOR: GREEN-RETURN, WHITE-DISCARD, RED-HAZARDOUS, BLUE-RUSH

SHEET DATA: PREPARE 2 COPIES IF1 TEST TYPE REQUESTED, 3 COPIES FOR 2 TYPES AND, 4 COPIES FOR 3 OR MORE TEST TYPES. TESTS MARKED WITH AN ASTERISK REQUIRE MAGNITUDE. CHECK MARKS OR MAGNITUDES SHOULD BE PLACED IN NARROW COLUMN. DESIGNATE METHOD.

RESERVE FOR ANAL. LAB.	240		223			
SAMPLE NO.	9A-358	9A-358	11-452	11-452	11-452	
TEST / METHOD	B-2	B-3	1-2	3-4	5-6	
ACID SOLUBILITY, UTM 87	12	12	10	10	10	
ANILINE PT. D611 50/50 OR MIX						
BR. NO. (ELECTRO) MCTL	2	2	2	2	2	
COLOR D155 D158						
CORR. D130 122° (212°)			✓	✓	✓	
D130 AIR WELL CU-DISH						
DIOLEFIN UTM 65						
DOCTOR D484						
DIST. (D86) 5% BOTTS	✓	✓				
D216 D158 RTM 69						
F.I.A. UTM 185	✓	✓	✓	✓	✓	
GRAVITY D287 PYC.			✓	✓	✓	
GUM CU-DISH GL-DISH						
D381 STEAM JET						
D873						
INDUCTION PERIOD D525						
KAURI BUTANOL UTM 94						
NITROGEN KJEL. DUMAS	.001	.001				
BASIC RTM 21						
PEROXIDE NO. UTM 44						
PONA RTM 67						
PHENOLICS U-V						
SULFUR P-BOMB O-BOMB						
(LAMP)	.01	.01	.005	.005	.005	
ELEMENTAL POLAR						
H_2S UTM 102						
MERCAPTAN UTM 102						
T.E.L. GRAV. POLAR						
VAPOR PRESSURE UTM 192						

Figure 7. Analytical request and report form

The form shown in Figure 7 is used for light oils. All forms have the same format and differ only in the tests that are listed in the bottom portion. The upper portion shows the type of information that is required on every sample.

Sample Tags. Tags are required on all samples, with client's name, date, and sample number or identification clearly indicated. The color is significant: manilla denotes that the sample may be discarded after completion of work and green that it is to be returned to the client; blue tags indicate rush work and red, hazardous materials. Blue or red tags are never used alone but in combination with routing tags.

Compartment Tags. The compartment tag, shown in Figure 8, is divided into one large and 21 small numbered spaces. The small squares are used by the clerk to indicate the number of tests to be performed on the sample and the large space is for the compartment number. This tag is the key to the sample handling system and has the following functions: It provides a simple way for the clerk to assign space; it tells the analyst where the sample should be stored and when to dispose of it; and it is returned to the clerk when tests are completed to indicate that the compartment is empty.

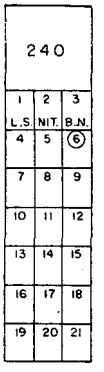


Figure 8. Compartment tag

Work Sheets. The recording of pertinent analytical data by the analyst is another operation that was studied carefully. All too frequently the analyst spends a considerable portion of his time transcribing information from the request form to work books, report sheets, and various other records with considerable chance of error in transcription. Printed work sheets have been adopted as providing the simplest and most economical means of recording these data. These sheets are mimeographed, as this permits rapid changes as methods are modified and allows the addition of new work sheets without undue cost.

The format of the work sheet is very important. The name of the test is printed at the top and where calculations must be made, an outline is given of the computation together with necessary factors. Although it is desirable to provide space for as many determinations as possible on the lower part of the sheet, overcrowding must be avoided if the record is to be legible. When determinations are run in sets, as, for example, lamp sulfurs, all are shown on one sheet, if possible. Spaces are provided for pertinent data, such as client, project number, sample designation, gravimetric and volumetric measurements, blanks, and normalities of solutions. A work sheet for a Kjeldahl nitrogen determination is shown in Figure 9.

Printed work sheets have several advantages. Analytical data are recorded only once and, if desired, a carbon copy may be retained by the analyst. Minimum writing by the analyst is required to enter complete data because data are placed in prepared spaces that show the source. Laboratory leaders may readily check calculations and note the quantity of work performed each day. Completed work sheets may be used by the card clerk to remove test cards from the active card file upon completion of the analysis.

Test Cards. The application of punched cards to analytical laboratory operations offers a time-saving method of storing information and making it readily available. The cards used in this laboratory are 3.3 × 7.5 inches and have five holes per inch.

They are obtained from the McBee Co. and are preprinted to indicate the data to be entered. Test and unit number information is mimeographed and prepunched for the more common tests in order to expedite the preparation of cards from the request sheet. These cards are filed in the bin shown in Figure 2 where they are readily accessible. Figure 10 shows a typical test card which has been punched to record the following information:

Data	Section	Code
John Doe	Chemist	271
Ash	Test	6
Rush sample	Priority	A
Project 162-26	Project	162-26
	Lab unit	23
	Service	2
	Completed	12

The first four lines of the tabulation are self-explanatory; the fifth shows that the analysis was made by the inorganic unit, a part of the chemical section; the sixth that it was completed in 2 days, and the seventh that it was completed in December.

International Business Machines equipment is being investigated for test accounting, as it is believed that the work load of the laboratory may now justify its use.

Time Sheets. Analytical labor is charged directly to the project for which work is done and personnel show this on their time sheets. Analysts are also required to indicate the type of labor performed and the number of the laboratory unit to which work is assigned. These classifications are described in the following tabulations:

Labor Types

Chemist	Technician	
161	181	Supervision and training
162	182	Sample handling and records
163	183	Calibration and maintenance
164	184	Sample analysis
165	185	Analytical research
166	186	Cooperative work

Laboratory Units

Chemical section		Spectral section		X-ray section	
Hydrocarbon	21	Emission spectrograph	31	X-ray	41
Electrometric	22	Mass spectrometer	32	Electron microscope	42
Inorganic	23	IR and UV spectrophotometer	33	Differential thermal analysis	43
Physical testing	24	Electronics	34		

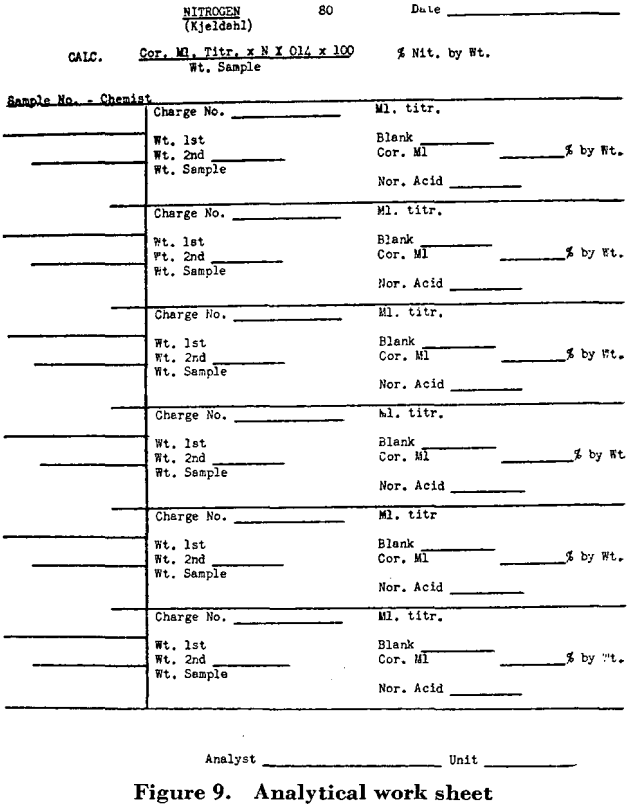


Figure 9. Analytical work sheet

Vacation, holiday, sick leave, and the time of the coordinators is not charged to laboratory units but to special accounting numbers. This information by type of labor and by laboratory unit within the analytical group has been most helpful in correlating time charges with work completed and may be readily understood by referring to the monthly operational report described in the preceding section.

Sample Receiving Office. The sample receiving office is near the center of the analytical building. Two walls of this room are formed by steel cabinets with sliding doors on each side. This arrangement permits the clerk to place samples in compartments from inside the room and the analyst to obtain them from the outside. Shelves in these cabinets are divided into compartments by egg case–type separators about 3 inches high, and the individual compartments are numbered on both sides. Adjacent to the storage cabinets are pigeonholes where work requests are filed by test for the analysts. The room also contains a table for incoming samples, the Thermo-Fax duplicator, the test card bin, and necessary desks and filing cabinets.

1. Issuing information to clients on procedures for making use of analytical services and to analysts on preparation of time and work sheets.
2. Liaison between clients and analysts on all matters pertaining to the handling, routing, and accounting of samples.
3. Continuous evaluation of methods for sample handling, record keeping, and preparation of statistical reports to ensure that improvements are made whenever possible.
4. Recording and transmitting requests for special analyses and analytical research to the Committee on Analytical Research and Development and providing a list of uncompleted projects for supervisory personnel.
5. Maintaining control on accuracy by obtaining data from analysis of standard samples, cooperative testing, and periodic rerunning of regular samples.
6. Calibration of equipment.

Methods. The coordinator of methods is responsible for maintaining an active file of test methods, distributing methods and related general information, and ensuring that the proper format is used for procedures written by analytical group personnel. This includes the distribution of analytical methods throughout

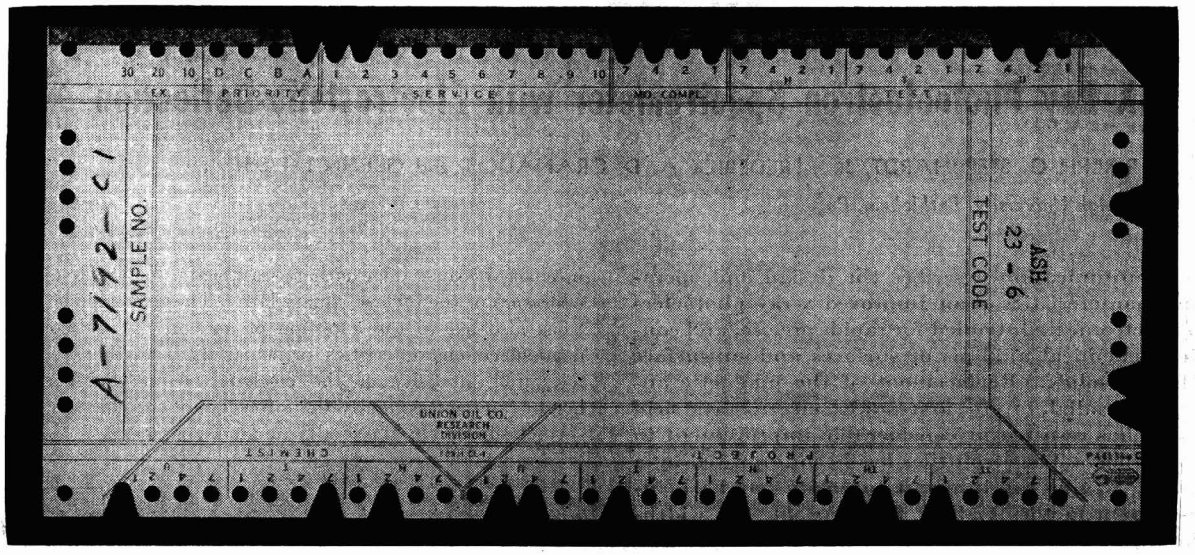


Figure 10. Test card

Duplicator. A Thermo-Fax duplicator, procured from the Minnesota Mining and Manufacturing Co., is used to prepare facsimiles of work requests, so that each analyst assigned to work on a sample will have a copy. The client supplies up to three copies; therefore, only about 10% of the requests require duplication. The duplicator (Figure 1), using the original request as a master, produces a positive, dry copy in about 10 seconds on special infrared-sensitive paper. Room lights need not be subdued and no solutions are required; however, black ink or soft lead pencil must be used in preparing the request.

Duplication of request sheets is considered worth while because it permits several tests on the same sample to be run concurrently, it provides the analyst with a complete, exact copy of the request, thus avoiding errors in transcription, and it allows each analyst to use his copy to report each test result as soon as it is obtained.

COORDINATORS

Sample Handling and Records. The coordinator of sample handling and records is responsible for preparing the reports discussed in this paper and for all operations of the sample receiving office and is also responsible for:

other departments of the company and preparation of memoranda on revisions of these methods. Digests are prepared of all commonly used methods to inform clients of the scope, limitations, analysis time, required sample size, precision, and accuracy.

The coordinator of methods also maintains a file of punched literature reference cards prepared by chemists of the analytical group. All analytical chemists are held responsible for reading and carding literature pertaining to petroleum analysis and specific assignments are made for topics of special importance. All correspondence of the American Petroleum Institute and the American Society for Testing Materials, company reports, and other pertinent unpublished documents are sent to these individuals for carding information on analytical procedures. This reference file is particularly useful for determining what information, related to specified subjects, is in the central research file. The coding and punching system is capable of expanding in size and diversity with the file and greatly increases the accessibility of this material.

CONCLUSIONS

The techniques and equipment described have proved to be efficient and economical in handling the normal flow of work in

the analytical group of the research and process department of the Union Oil Co. of California. Supervisory personnel and management are provided with statistical reports that give a comprehensive survey of the operations of the group and also permit realistic predictions of future requirements for space, equipment, and personnel. The expense of centralizing all paper work and movement of samples is more than compensated by savings in analyst's and client's time. Furthermore, more effective utilization of the training and experience of the analysts is possible.

These techniques should be effective in any analytical laboratory of over 30 persons that is serving a research group. Not all of the procedures would necessarily be advantageous, because the basic policies of another laboratory might be sufficiently different to make some procedures inefficient or even unnecessary. These techniques were not all developed in this laboratory; in fact, they are a composite of ideas from many laboratories.

This paper is presented with the hope that it will be provocative and stimulate greater interchange of ideas on the subject. No claim is made that this is the best plan, even for this laboratory, but it is hoped that it will encourage readers to get in contact with the authors and exchange ideas *for mutual benefit.*

ACKNOWLEDGMENT

The authors wish to express their appreciation to G. R. Lake for his interest and guidance in the preparation of this paper and to the other members of the group for their helpful suggestions. They are also thankful for the information and inspiration gained from contacts with many other laboratories. Permission of the Union Oil Co. of California to publish this paper is gratefully acknowledged by the authors.

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X-Ray Photoelectron Spectrometer with Electrostatic Deflection

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This communication describes the design and operational characteristics of an improved x-ray photoelectron spectrometer intended for rapid, precise, and convenient chemical analysis of surfaces and subsurface regions of solids. Replacement of the magnetic analyzer by a radial, inverse first-power, electrostatic field analyzer has resulted in considerable improvement in performance. The resolution has been improved by a factor of 5, and the inherent intensity has been increased by a factor of about 20. Variable slit widths make it possible to vary the resolution by a factor of 8, leading to a considerable potentiality for versatility. Other refinements decrease the time of a routine determination from 2 to 4 hours to less than 10 minutes.

THE initial work on the chemical applications of x-ray photoelectron emission spectra (*10*, *11*) was carried out using a homogeneous-field, 180° deflection, magnetic analyzer for electron dispersion. This type of instrument was chosen originally because it appeared unwise to build a complicated and expensive instrument with which to carry out exploratory work. However, as the need for better precision and greater rapidity grew with the analytical potentialities of the method, the limitations of the magnetic deflection instrument became more evident.

Objections to the magnetic analyzer stemmed directly from the use of an iron electromagnet. The hysteresis associated with operation at low magnetic-flux intensities led to considerable difficulty in obtaining accurate electron energy measurements and necessitated time-consuming operations to minimize its effects. To avoid fringe effects of the field, it was necessary to extend the pole faces well beyond the limit of the spectrometer chamber and to keep their separation as small as possible. This meant that the x-ray tube had to be placed relatively far from the sample, leading to a considerable loss of potential photoelectron intensity because the exciting x-ray beam was weakened through operation of the inverse-square law. These geometric considerations also led to the adoption of an end-window counter, the dead-space characteristics of which (*12*) further reduced the measured intensity of the electron beam. Furthermore the height (and consequently the intensity) of the electron beam was severely limited by the small pole face separation.

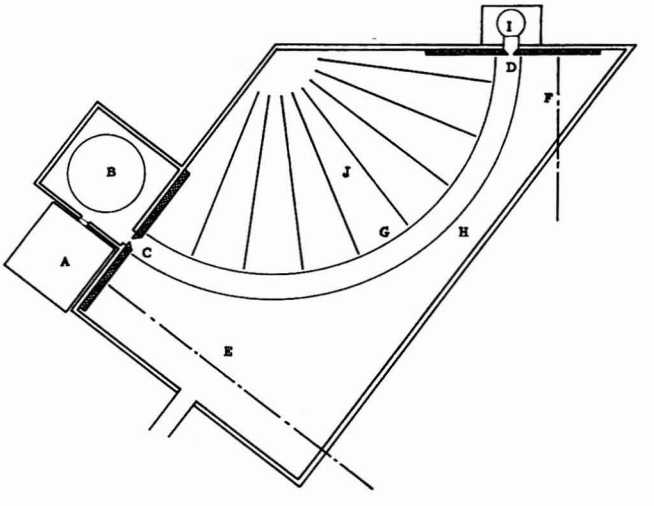

Figure 1. Schematic diagram of spectrometer chamber

The criteria which were established for the characteristics of a new analyzer were the following:

1. The resolution should be conveniently variable from $\frac{\Delta E}{E} \cong 0.005$ to 0.025.
2. The best accuracy of energy evaluation should be of the same order of magnitude as the best resolution—i.e., 0.5%.
3. The inherent intensity should be sufficiently great to allow rapid determinations with good intensity precision.

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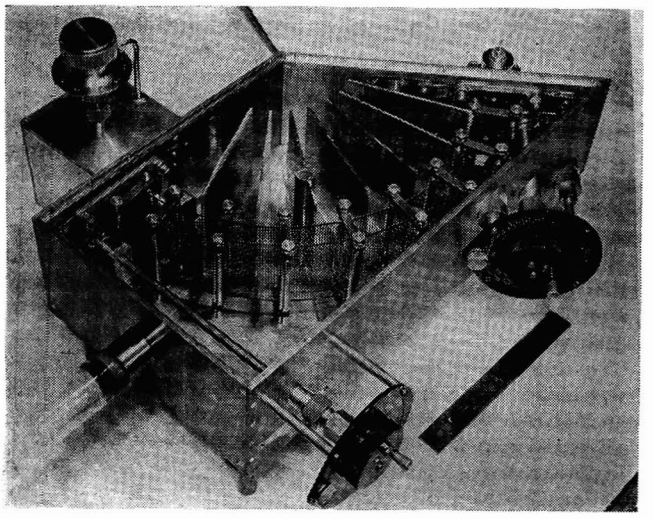

Figure 2. Interior view of spectrometer chamber

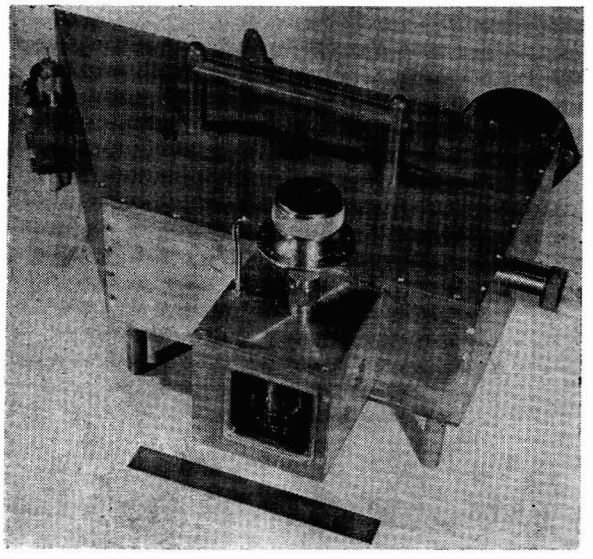

Figure 3. Exterior view of spectrometer chamber

4. Provision should be made for rapid change of samples, either by vacuum lock or by multiple mounting.
5. The apparatus should be reasonably compact and capable of being used conveniently with commercially available x-ray sources.

Extensive consideration of many designs of electron spectrometers (*8*) led to the conclusion that a 127° 17′ deflection electrostatic analyzer would make it possible to fulfill the above criteria. Not only does this type of instrument eliminate the possibility of hysteresis effects, but its simplicity of design avoids severe geometric and orientational difficulties which other types entail.

THEORY

The theory of operation of the radial, inverse first-power, electrostatic field as a means of analyzing electronic velocities has been given by Hughes and Rojansky (*7*), who showed that in such a field quasi-refocusing of an initially divergent beam of electrons occurs at a deflection of 127° 17′ and that the resolution is best at this angle. Consider two coaxial cylindrical electrodes, one negatively and the other positively charged, and with radii r_- and r_+ respectively. If $r_- > r_+$, and the potential difference between the electrodes is ΔV, an electron of energy E will follow a circular path of radius $r_0 = (r_- + r_+)/2$, if

$$E = \Delta V \frac{r_0}{2\Delta r} \tag{1}$$

in which $\Delta r = r_- - r_+$. The resolution for this energy is

$$\frac{\Delta E}{E} = \frac{w_s}{r_0} \tag{2}$$

where w_s is the width of a slit located at 127° 17′. As the entire range of the spectrometer involves electronic velocities which are not small compared with that of light, it is necessary to introduce a relativity correction and thus to modify Equation 1. Rogers (*9*) has developed the relativistic equation for the radial, inverse first-power electrostatic field spectrometer. On the basis of this work, the relativistic analog of Equation 1 is:

$$E = kmc^2\left[\left(1 - \frac{2\Delta V\frac{r_0}{2\Delta r}}{kmc^2}\right)^{-1/2} - 1\right] \tag{3}$$

in which

$k = 0.6246 \times 10^{12}$ ev./per erg
$m = 0.9107 \times 10^{-27}$ gram (mass of electron)
$c = 2.9978 \times 10^{10}$ cm./per second (velocity of light)

DESIGN AND CONSTRUCTION

The electrostatic analyzer is shown diagramatically in Figure 1 and is illustrated in Figures 2 to 4. In some respects the instrument is similar to that of Backus (*1*). The chamber is constructed of 0.25-inch rolled brass; the walls, bottom, and top are fitted together with step joints to provide a reasonably long leakage path. High-vacuum wax is used instead of gasketing to prevent leaks at the joints, and the entire chamber was painted with Glyptal. A pressure of 5×10^{-5} mm. of mercury was routinely obtained using a DPI GF-25 diffusion pump (Distillation Products, Industries, Rochester, N. Y.).

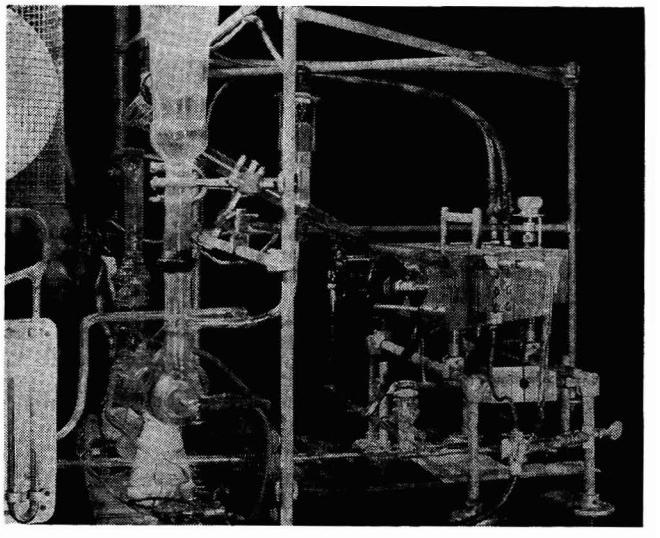

Figure 4. Spectrometer chamber in place

Samples are mounted on a drum (*B*, Figure 1), which may be rotated from outside by a shaft running through a Wilson seal. An index is provided so that any chosen sample may quickly be brought into operating position without opening the chamber to the atmosphere. As many as 48 samples may be mounted on the drum at one time. This arrangement greatly reduces the average time necessary for analysis.

The sample is irradiated with a zirconium-filtered beam of a Machlett 0–2 molybdenum target x-ray tube, *A*, operating at 50 kv.p. and 10 ma. Beryllium windows on both the tube and the chamber make it possible to bring the beam into the spectrometer without excessive absorption, and an external lead slit limits the size of the irradiated area of the sample. Because of the lack of interference from an overhanging magnet it is possible to bring

the focal spot of the tube to within 3 cm. of the sample rather than 7 cm. as previously required. This provides an inherent intensity increase factor of 5.4.

The irradiated area of the samples is twice as great as in the magnetic analyzer, the increase being brought about by doubling the height of the irradiated region. This increases the inherent intensity by an additional factor of 2 without the sacrifice in resolution that an increase in width would entail. The effect of a new counter design, also made possible by the elimination of the magnet, is a further factor of 2. Thus the removal of the geometric restrictions imposed by a homogeneous magnetic field have made it possible to increase the measured intensity of the beam by a factor of about 20 without loss of resolution. This makes it possible to improve the precision of intensity determination by about 4.5 without increasing the length of the counting periods.

The entrance and exit slits, *C*, *D*, are externally and reproducibly adjustable by means of Wilson seals, *E*, *F*. Adjustment of the width of the entrance slit allows the attainment of the maximum possible intensity for a given resolution, and adjustment of the exit slit makes it possible to vary the resolution itself from its best value of 0.003 to its poorest of 0.025. (The resolution of the magnetic spectrometer was 0.014 at best and was not externally adjustable.) The slits are constructed of Bakelite to reduce electron scatter at the edges. (Although Bakelite is not ordinarily advised for use in high vacuum, it was found satisfactory in this case. No difficulty was encountered, presumably owing to careful vacuum cleaning and pretreatment of the Bakelite parts and to the use of a fast diffusion pump.)

The deflection electrodes, *G*, *H*, are made of bronze gauze, 10 × 0.010 inches. The wide mesh and fine wire combined with the effect of a colloidal graphite coating minimize electron scatter by the electrodes. All edges of the gauze are beaded, and the electrodes are supported and located by glass insulators and Bakelite spacers. The height of the electrodes is three times the height of the electron beam; this considerably reduces distortion of the beam by edge effects. The radii of the electrodes are $r_- = 16.0$ cm. and $r_+ = 14.5$ cm. A variable deflection potential is supplied by a 0.1% precision, regulated power supply designed by Higinbotham (*5*) and modified to give a center-ground output. The spectrometer chamber and the ground of the power supply are connected, thus providing a ground potential for the center of the electron beam with a radius r_0. The stability and precision of the Higinbotham circuit make it possible to obtain energy values accurate to about 0.5% and with precision to about 0.1%. (The power supply is built on three separate, transparent plastic chassis. This serves the purpose of eliminating electrical leakage, and makes construction and maintenance extremely convenient.)

The intensity of the beam is measured with an ultra thin-window Geiger-Müller counter (*I*). A side window rather than the previously used end window is employed. Besides providing a larger aperture, this counter does not present a dead space to the electron beam. The net result of this modification is the twofold increase in intensity noted above.

To prevent electrons of other than a particular selected energy from reaching the counter, thin aluminum baffles, *J*, are mounted vertically and oriented radially at intervals of 15° on the positive side of the deflection electrodes. Also, the interior of the chamber is lined with aluminum on the negative side of the electrodes. In this manner scattering and electron re-entrance along the positive electrode is minimized.

All other aspects of the instrument have been described (*10*, *11*).

EXPERIMENTAL RESULTS AND DISCUSSION

Calculations of theoretical values of the various interactions referred to in this section have been made using $V\lambda = 12{,}370$ volt kx-unit (Siegbahn) as given by Felt, Harris, and DuMond (*4*). Energies of absorption levels were obtained from the recent summary by Hill, Church, and Mihelich (*6*), and wave lengths of emission lines from the values given by Compton and Allison (*2*).

Energy data are obtained from the analyzer in terms of the potential difference, ΔV, between the electrodes. To convert these measurements to energy values, in electron volts, Equation 3 is used. Preliminary results showed, however, that Equation 3 did not give results to better than −2% at the upper energy limit. It was found that $r_0/2\ \Delta r$ appeared to vary slightly as a linear function of the energy, presumably as a result of electrode edge effects. Because of the linearity of the function, calibration of the instrument was carried out by evaluating $r_0/2\ \Delta r$ in Equation 3 using the theoretical values of MoKα_1–AuL$_{III}$, MoKα_2–AuL$_{III}$, and MoKα limit. Once the spectrometer had been calibrated in this manner, reproducible and accurate energy measurements could easily be made, as the power supply contains a calibration circuit with which an accurately known fraction of the deflection voltage may be compared with the output of a standard cell.

The spectra of two very pure metals, gold and rhodium, were used to study the operational characteristics of the electrostatic analyzer. Gold was chosen because of the richness of its spectrum in Auger and externally excited peaks, and rhodium because of the simplicity of its spectrum in the present range of the spectrometer.

The spectrum of rhodium at $\Delta E/E = 0.008$ is shown in Figure 5. Only two major peaks are noted. The peak at 14.70 k.e.v. is the MoKα–RhL interaction and that at 17.40 k.e.v. is caused by the interaction of MoKα quanta with lower rhodium levels. Because of the very small differences in energy of the lowest levels regardless of the nature of the sample, the peak at 17.40 appears in all MoKα-excited spectra. This is designated as the MoKα limit. The minor peak at about 10.50 k.e.v. also appears in most spectra and corresponds to the PbLα limit. The fluorescence of the lead slits excited by the primary x-ray beam is sufficiently intense to cause the appearance of this small peak.

The spectrum of gold at $\Delta E/E = 0.008$ (Figure 6), in contrast to the rhodium spectrum at the same resolution, shows a number of prominent peaks. Most of these are Auger peaks; only three

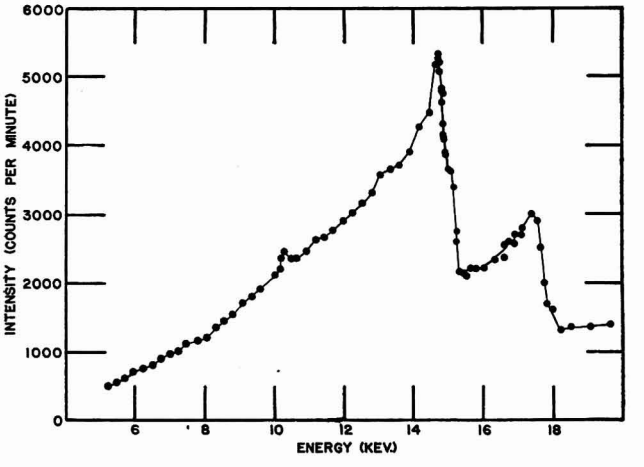

Figure 5. X-ray photoelectron spectrum of rhodium

$\Delta E/E = 0.008$

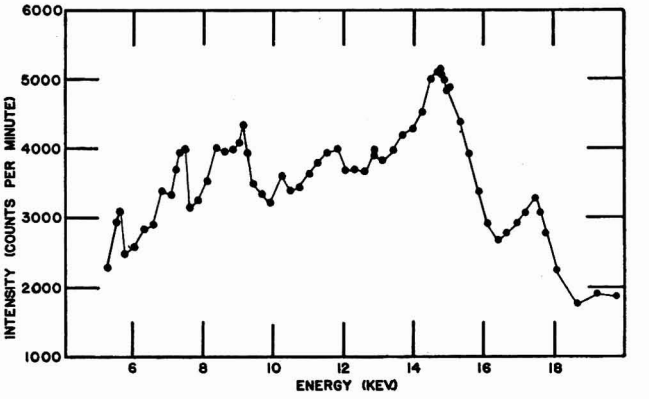

Figure 6. X-ray photoelectron spectrum of gold

$\Delta E/E = 0.008$

of the 10 peaks are excited directly by the MoKα radiation. Table I identifies the peaks in the gold spectrum. In all cases the observed Auger peaks are those which are to be expected on the basis of the relative intensities of the AuL group emission lines (*3*).

That a considerable amount of spectrum detail is retained at poorer resolutions is indicated by comparing Figure 6 with Figures 7 and 8. In the latter, gold spectra are shown at $\Delta E/E = 0.017$ and 0.025, respectively. In all three cases, counting periods were chosen so that the precision of intensity determination was $\sigma = 2\%$. The times of determination of the spectra were $\Delta E/E = 0.008$, 75 minutes; 0.017, 20 minutes; 0.025, 7 minutes. It is apparent that the retention of detail in low resolution spectra makes it possible to determine routine spectra in unexpectedly short periods of time.

A portion of the spectrum of gold at highest resolution ($\Delta E/E = 0.003$) is shown in Figure 9. The first peak is the MoKα_2–AuL$_{III}$ and the second is the MoKα_1–AuL$_{III}$. The separation of these peaks is calculated on the basis of crystal spectroscopic data as 0.1049 ± 0.00004 k.e.v. X-ray photoelectron data yield an average value of 0.100 ± 0.009 k.e.v. The precision of energy determination here is somewhat greater than the design figure of 0.1%. This, of course, is the result of using an average of several determinations. Single determinations of energy values are usually of the expected order of about 0.1%. Although the accuracy of the determination of MoKα_1–MoKα_2 is only about 10%, this value is determined by a difference calculation. For direct evaluations, an accuracy of about 0.5% can usually be attained at highest resolution. The ability of the instrument actually to measure the MoKα_1–MoKα_2 separation with reasonable accuracy indicates that further improvement in resolution will not increase the utility of the instrument in chemical applications.

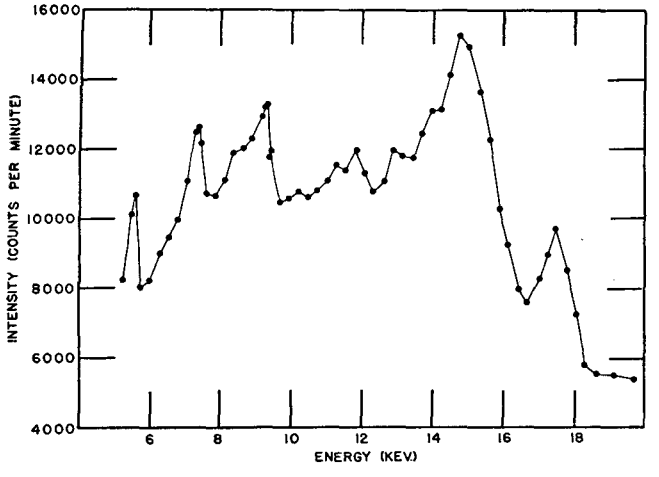

Figure 7. X-ray photoelectron spectrum of gold

$\Delta E/E = 0.017$

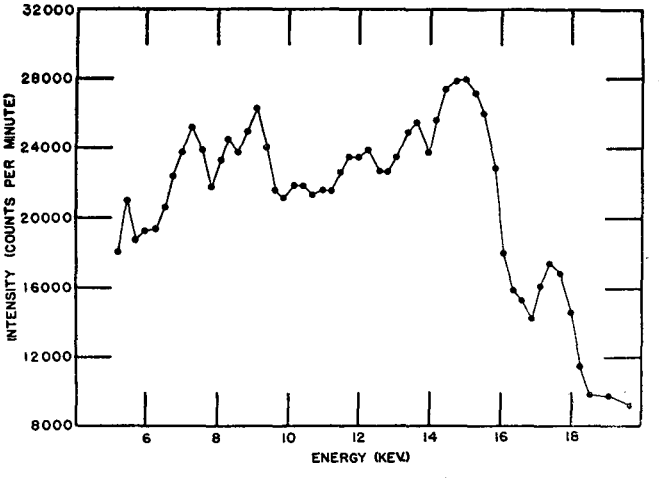

Figure 8. X-ray photoelectron spectrum of gold

$\Delta E/E = 0.025$

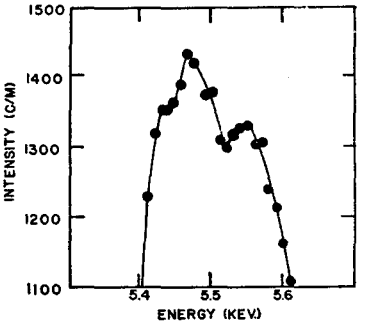

Figure 9. Detail of x-ray photoelectron spectrum of gold

$\Delta E/E = 0.003$

Determinations of differences in the chemical nature of samples may be made by comparing their intensity ratios as a function of energy. If the samples are identical, the ratio remains constant; if they are different, the ratio varies considerably. This method is valid even if the samples have different roughness values. Three samples of gold were used to validate this parallel method of determining chemical similarity and differences. Two of the samples (*A* and *B*) had almost identical roughness values (approximately 2 microinches root mean square), and the third (*C*) was considerably different from the others (approximately 15 microinches root mean square). The results are indicated in Table II. It will be noted that the observed standard deviation for *A*/*C* and *B*/*C* are of the same order of magnitude as that for *A*/*B*, indicating that differences in roughness do not affect the intensity ratio. The actual standard deviation for a single comparison is from about 20 to 30% higher than the theoretically probable value of the standard deviation. The latter value is based on a purely random process. The consistently higher value for the actual standard deviation is caused by small variations in x-ray intensity. As the observed ratios for chemically different samples vary strongly as a function of energy, the

Table I. Observed Peaks in Typical Photoelectron Spectrum of Gold[a]

Mechanism	Observed Energy, K.e.v.	Theoretical Energy, K.e.v.
MoKα–AuL$_{III}$	5.65	5.51
AuLα_1–AuM$_{II}$	6.75	6.57
AuLα_1–AuM$_{IV}$	7.40	7.42
AuLβ_1–AuM$_{II}$	8.30	8.44
AuLα_1–AuN$_{II, III}$	9.05	9.07, 9.17
AuLβ_1–AuM$_{IV}$	9.05	9.15
PbLα limit (?)	10.15	10.50
AuLβ limit	11.75	11.61
AuLγ_1–AuN	12.80	12.86 (av.)
MoKα–AuM	14.70	14.64 (av.)
MoKα limit	17.40	17.42

[a] $\Delta E/E = 0.008$.

Table II. Intensity Ratios of Spectra of Three Samples of Gold

	A/*B*	*A*/*C*	*B*/*C*
Average intensity ratio	0.997	0.830	0.833
Std. dev. of single comparison, %			
Observed	3.35	3.05	2.80
Theoretical	2.74	2.29	2.29

difference between the actual and theoretical standard deviations may be considered negligible.

ACKNOWLEDGMENT

The authors wish to express their deep gratitude to Willard S. Clewell, Sr., whose superb craftsmanship in the construction of most of the parts of the analyzer contributed greatly to the work described here; to Beckman Instruments, Inc., South Pasadena, Calif., for its financial support of the x-ray photoelectron spectrometer program; and to Earl J. Serfass for his generous interest and valuable help.

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Spectrophotometric Determination of Cerium

After Oxidation to Cerium(IV) with Lead Dioxide

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The instability of dilute solutions of cerium(IV) presents a limitation on spectrophotometric methods based on the cerium(IV) color. Other limitations arise because of interfering substances which absorb in the same spectral region as does cerium(IV). Such substances may also include the excess oxidant used to convert cerium to the quadrivalent state and are often difficult to remove. In the present method cerium(III) is oxidized to the quadrivalent state by lead dioxide in sulfuric acid medium. Excess lead dioxide is easily separated from the unstable ceric solution, which is immediately reacted with excess ferrous ammonium sulfate. The latter is subsequently treated with an excess of *o*-phenanthroline and compared to a cerium-free blank prepared in the same manner. From 20 to 1000 γ of cerium can easily be determined. The method is simple, rapid, and accurate. It permits the determination of cerium in the presence of such interferences as thorium and the rare earths. The general procedure should be applicable to other elements.

IN THE course of a coprecipitation study it was found desirable to have a method for the determination of small quantities of cerium. Many of the methods which have been proposed (*3, 5, 7, 10*) are based on the measurement of the cerium(IV) color intensity in the ultraviolet region of the spectrum. However, dilute solutions of cerium(IV) are unstable (*12*). Furthermore, excess reagent used to oxidize cerium(III) is often difficult to remove and may also absorb in the ultraviolet region, as do thorium, the rare earths, and many other common ions. Methods based on the measurement of ceric perhydroxide (*1, 10, 13*) suffer from the same disadvantages as above and because of the many interferences require preliminary separations. A number of other reagents such as brucine (*10*), and gallic acid (*11*), have been suggested for this determination, but have not found wide application.

In the present method, cerium(III) is oxidized to cerium(IV) with lead dioxide in sulfuric acid media. The cerium(IV) is then made to react with excess iron(II) immediately after excess oxidant is removed by filtration. The residual iron(II) is determined spectrophotometrically with *o*-phenanthroline, and compared to a cerium-free blank.

EXPERIMENTAL

Reagents and Apparatus. Ammonium hexanitrocerate (standard of reference purity), G. F. Smith Chemical Co. This salt was converted to cerous sulfate or cerous perchlorate. It was first treated with hydrochloric acid to reduce the cerium to the trivalent state. Sulfuric or perchloric acid was then added and the solution was evaporated to dryness to remove ammonium, nitrate, or chloride ions. The residue was dissolved in 0.5*N* sulfuric acid. Solutions were standardized gravimetrically by the oxalate procedure and volumetrically by titration with standard ferrous sulfate after oxidation with persulfate. Less concentrated solutions were prepared by dilution.

Ferrous ammonium sulfate (reagent grade) Baker and Adamson. A solution containing 4.2 grams of ferrous ammonium sulfate per liter of 0.5*N* sulfuric acid was prepared; dilute solutions were freshly prepared as needed. For 0 to 150 γ of cerium, 5 ml. of this solution were diluted to 100 ml. For larger quantities of cerium, up to 20 ml. were diluted.

o-Phenanthroline monohydrate, G. F. Smith Chemical Co. One gram was dissolved in water at 80° and the solution diluted to 1 liter.

Lead dioxide (manganese- and chloride-free) Baker's analyzed.

Thorium nitrate, Code 103 (cerium maximum 0.0001%), Lindsay Chemical Co. This was converted to the perchlorate and dissolved in water.

Yttrium, neodymium, lanthanum, praseodymium, and samarium oxides (>99.9% pure). These were dissolved in perchloric acid.

Beckman spectrophotometers, Models B and DU, with 1-cm. silica and borosilicate glass cuvettes.

Sintered-glass fiber filtering disks, Hurlbut Paper Co.

Choice of Oxidizing Agent. Several oxidants are used for the oxidation of cerium(III). Sodium bismuthate (*10*) is not satisfactory because bismuth interferes in the iron *o*-phenanthroline method. Anodic oxidation proved difficult to control. Perchloric–sulfuric acid media will oxidize cerium(III), but the time interval required for cooling and dilution before addition of iron(II) is sufficient for some reduction of cerium(IV) to occur. From a consideration of the electrode potentials (*4*) of the half reactions:

$$Ce^{+++} \rightarrow Ce^{++++} + e \qquad E_0 = -1.44$$

$$PbSO_4 + 2H_2O \rightarrow PbO_2 + 4H^+ + SO_4^{--} + 2e \qquad E_0 = -1.67$$

and other factors, it appeared that lead dioxide would serve as a useful oxidation agent. It is insoluble in the media used as is the lead sulfate formed so that both may easily be removed by filtration. Job (*6*) previously suggested the use of lead dioxide and reported the oxidation to be instantaneous and complete in strong nitric acid. However, because concentrated nitric acid will oxidize iron(II) to iron(III) in the present work sulfuric acid was substituted with excellent results. From 20 to 1000 γ of cerium were treated with 0.3 to 0.5 gram of lead dioxide in solutions 0.5 to 6*N* in sulfuric acid. The reaction was found to be quantitative in this acid range. One to 2*N* acid was subsequently used, so that excessive amounts of ammonia would not be required for later neutralization.

Batch treatment of cerous solutions with lead dioxide was found to be effective. Satisfactory separation of the solution and solid phases was achieved with the use of a sintered-glass fiber filtering disk in conjunction with a Royal Berlin filtering crucible, porosity A-3. The use of a filter disk permitted the same crucible to be used for eight to ten successive filtrations without clogging.

Color Reaction. Freshly oxidized samples of cerium were found to decrease in absorbancy rapidly. If kept in the dark, the solutions were more stable but decomposition still occurs. Introduction of large amounts of sulfate to complex the cerium and thus stabilize the solution was found to be ineffective. As cerium(IV) reacts quantitatively with iron(II) and the latter is easily measured by the *o*-phenanthroline method (*2*), this system was therefore employed as a measure of cerium.

Comparison of the absorption spectra of solutions containing iron *o*-phenanthroline and cerium(III) with an equivalent amount of ferric ion showed the same peak in the 505-mμ region as is shown by the iron *o*-phenanthroline system. Although the color intensity of the iron *o*-phenanthroline is independent of pH in the range 2 to 9 (*2*), solutions were adjusted to pH 2.5 to 2.8 with ammonia or sulfuric acid in order to prevent precipitation of the hydroxides of cerium, other rare earths, or thorium. Color development is complete in 30 minutes at this pH (*9*). The color is stable for at least 72 hours beyond this.

The solutions of oxidized cerium were filtered directly into a measured excess of ferrous ammonium sulfate which was subsequently treated with an excess of *o*-phenanthroline. The pH of the solutions was adjusted and the latter were diluted to 100 ml. in volumetric flasks. A similarly treated solution without cerium was also prepared. The difference in intensity of the two solutions is directly proportional to the amount of cerium present. Untreated blanks containing cerium were identical to cerium-free blanks which had been treated by the oxidation procedure. However, it is recommended that this be verified for each batch of lead dioxide. Samples containing highly colored substances may thus be determined by using the sample itself as a blank.

RECOMMENDED PROCEDURE

To 20 to 1000 γ of cerium, chloride-free, add 2 to 4 ml. of concentrated sulfuric acid and adjust the volume to 10 to 25 ml. Add 0.3 gram of lead dioxide. Stir occasionally. After about 5 minutes filter through a filtering crucible fitted with a sintered-glass disk into 10.00 ml. of the dilute ferrous sulfate solution. Add 10 ml. of the 0.1% *o*-phenanthroline solution and sufficient ammonia to turn the solution red. Cool, then adjust the pH to 2.5 to 2.8, and dilute the solution to 100 ml. Prepare a cerium-free blank in the same manner. The difference in absorbancies measured at 505 mμ is proportional to the cerium content.

Using 1-cm. borosilicate glass cells and a standard cerium solution, a calibration curve was prepared following the recommended procedure. The change in absorbance was plotted against micrograms of cerium.

RESULTS AND DISCUSSION

The method was found to follow Beer's law over the range of cerium concentrations studied. The molar extinction coefficient was found to be the same as for the determination of iron with *o*-phenanthroline—i.e., 1.0×10^4.

The effects of thorium and some rare earths were studied with the results shown in Table I. A factor which must be considered when working with thorium is the limited solubility of thorium sulfate in sulfuric acid. Chloride must be absent, as well as ions which interfere in the iron *o*-phenanthroline method (*10*). Manganese and vanadium would also interfere.

Table I. Effect of Interferences

(Cerium taken = 92 γ)

Diverse Ion[a]	Ratio: $\frac{\gamma \text{ Diverse Ion}}{\gamma \text{ Cerium}}$	Cerium Found, Difference γ
La	454	±0, 1
Nd	228	±0, +1, +1
Pr	196	±0, −1
Sm	217	+1, +1
Y	44	+1, +1
Th	543	+2, +2, +1, +1

[a] All taken as perchlorates; thorium used as nitrate, sulfate, and perchlorate with identical results.

On the basis of the spectra of europium, gadolinium, erbium, thulium, and ytterbium, as reported by Moeller and Brantly (*8*), and terbium, dysprosium, and holmium as reported by Yost *et al.* (*14*), the rare earths would not be expected to interfere.

The method presented is simple, fast, and accurate. It is considerably more sensitive than other methods for cerium and is applicable in the presence of thorium and the rare earths. Its advantage over the persulfate and peroxide procedures lies in the ease of removal of excess oxidant and the greater stability of the colored substance measured, because the cerium(IV) which is formed is immediately reduced.

Preliminary experiments in this laboratory indicate the feasibility of oxidizing larger quantities of cerium by column treatment with lead dioxide. Subsequent determination can be completed by titration with standard ferrous sulfate solution. Furthermore, the same general procedure may be applied to the oxidation and determination of other elements—for example, manganese could be oxidized with lead dioxide and determined with iron *o*-phenanthroline, in a similar manner.

ACKNOWLEDGMENT

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Flame Spectrophotometric Determination of Iron in Siliceous Materials

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This investigation describes the application of the Beckman Model DU flame spectrophotometer to the rapid determination of iron in siliceous materials such as alumina refractories and limestone. The arc emission line at 386.0 mμ was used to measure the iron radiation. Cobalt was incorporated in the samples in order that the cobalt 387.1-mμ arc line could serve as an internal standard. An oxyacetylene flame was the excitation source. The radiative interference of many elements normally associated with iron in siliceous samples could be corrected by using an internal standard. None of the alkalies or alkaline earths offered interference when present in the amounts usually encountered, and large concentrations of aluminum can be tolerated. Magnesium offers serious interference, and when present, the separation of iron as the hydrous oxide is necessary. The optimum concentration range for iron is between 25 and 200 p.p.m. The standard deviation from the mean of replicate samples is approximately 3%. The method has been successfully applied to limestone and several varieties of alumina refractories.

THIS investigation describes the application of the flame spectrophotometer to the rapid, routine determination of iron in siliceous materials such as alumina refractories, glasses, and limestone. The method should be of particular interest to those laboratories which are desirous of a rapid method for the analysis of iron in these types of materials wherein the iron content embraces the concentration range of many colorimetric procedures.

Determination of iron by flame spectrographic methods has been discussed by Mitchell (*12*) and McClelland and Whalley (*11*), and has been applied to agricultural materials, water, and blood by Griggs (*7*), and Griggs, Johnstin, and Elledge (*8*). These workers used photographic recording in conjunction with a spectrograph. Gerber, Ishler, and Borker (*6*) have reported orally on the use of the Beckman flame spectrophotometer for the determination of iron. Therefore, a method employing the modern flame spectrophotometers would be desirable in addition to the restudy of some of the variables encountered when using the newer instruments and the integral atomizer-burners.

EXPERIMENTAL

Apparatus. A Beckman Model DU spectrophotometer with Model 9220 flame attachment and photomultiplier unit was used. An all metal atomizer-burner unit, supplied with the flame attachment, was used as the excitation source. The gases chosen were oxygen and acetylene, largely because of availability, although the slightly higher excitation energy available from the oxyacetylene flame, as compared with an oxyhydrogen flame, was a prominent consideration.

Reagents. A standard solution of iron, 1.00 ml. equivalent to 10.0 mg. of iron, was prepared by dissolving 10.0 grams of iron wire of known purity in 115 ml. of 6*N* hydrochloric acid and diluting to 1 liter with demineralized water. Weaker standard solutions were prepared by appropriate dilution with demineralized water. Sufficient hydrochloric acid should be added to the standard solutions to adjust the hydrogen ion concentration to approximately 0.3*N*.

A standard solution of cobalt, 1.00 ml. equivalent to 14.73 mg. of cobalt, was obtained from the Burrell Corp., Pittsburgh, Pa. A weaker standard solution, 1.00 ml. equivalent to 2.00 mg. of cobalt, was prepared by transferring 67.9 ml. of the foregoing solution to a 500-ml. volumetric flask, and diluting to the mark with demineralized water. The latter solution was employed for all samples composited with cobalt added as an internal standard.

Demineralized water, used exclusively in preparing all solutions, was prepared by passing ordinary distilled water through a bed of Amberlite MB-3 resin.

Flame Spectrophotometer Settings. The instrument settings used to measure the iron luminosity were as follows:

Sensitivity control, turns from clockwise limit	5 to 6
Selector switch	0.1
Phototube resistor, megohms	22
Slit, mm.	0.050
Acetylene, lb. per square inch	4.5
Oxygen, pounds per square inch	9 (burner rated at 10 pounds per square inch by manufacturer)

Different aspirator-burners, even though of similar construction, do not necessarily reproduce these tabulated luminosities when employing these operating conditions. Differences in diameters of the oxygen and capillary orifices, and tiny obstructions in or around the oxygen orifice, affect not only the flow of oxygen, but also the rate of aspiration of the solution under examination. These factors alter the flame temperature, and consequently, affect both the flame background and the iron luminescence. The burner should be cleaned frequently to remove the carbon deposits which tend to accumulate.

Flame Spectra of Iron. The flame spectra of iron were determined by aspirating an aqueous solution containing 100 p.p.m. of iron into the flame. Figure 1 gives the major emission lines of iron that are found in the outer flame mantle in the region suitable for the flame photometric determination of iron.

For quantitative analysis an iron line is needed which possesses sufficient luminosity to enable iron to be determined in refractory type materials wherein the iron content ranges between 0 and 200 p.p.m. Although this study revealed numerous iron lines in this region, only four emission lines possessed sufficient intensity to be considered suitable for quantitative analysis. Table I gives the wave length of each of these four lines, together with the relative intensities of each. The 374-mμ peak is actually an unresolved grouping consisting of three closely spaced lines for iron 374.56, 374.59, and 374.8 mμ. Such an unresolved grouping is unsuited for quantitative work.

Of all the emission lines the 372.0- and the 386.0-mμ lines were chosen initially for investigation because they were more sensitive. Study of the 372.0-mμ line was soon abandoned when this line was found to suffer severe interference from strong magnesium

Table I. Major Flame Emission Lines of Iron

Wave-Length Line Peak, Mμ	Relative Intensity of Line, P.P.M. of Iron[a]
372.0	1.0
373.7	1.8
374.7	2.5
386.0	1.3

[a] Expressed as minimum quantity detectable for a slit width of 0.050 mm.

band systems occurring between 367 to 373 mμ. The 372.0-mμ iron line suffers a further limitation in that it is a resonance line—that is, its emission arises from an electron falling back to the lowest energy level (ground state) from the next upper electron level. A resonance line is more prone to exhibit self-absorption than are other spectral lines of the same element. For these reasons, subsequent efforts were confined to the 386.0-mμ line.

Also noticeable in Figure 1 are several very weak iron emission lines. For slit widths of 0.050 mm., approximately 16 discrete iron emission lines can be resolved from 335 to 395 mμ (*9*). The remainder of the background radiation in the vicinity of the iron lines is essentially continuous and is attributable to the continuous spectrum of the carbon monoxide flame and the superposition of innumerable feeble iron lines.

OPTIMUM INSTRUMENT SETTINGS

Fuel and Oxygen. The pressures chosen were 4.5 pounds per square inch of acetylene and 9 pounds per square inch of oxygen for a relatively wide orifice burner, and 5 pounds per square inch of acetylene and 18 pounds per square inch of oxygen for a burner with a narrow orifice. These burners were rated at 10 and 19 pounds per square inch of oxygen pressure, respectively, by the manufacturer. The gas pressures were arrived at by varying the oxygen and acetylene pressures independently. Although higher acetylene-oxygen ratios gave larger luminosity readings, the background also increased proportionately and there was little over-all gain in sensitivity. These pressures were used throughout the remainder of the investigation because the ensuing flame permitted iron to be determined with the desired sensitivity.

Slit Width. The slit width used was 0.050 mm. At this slit width the effective line width of the iron 386.0-mμ line and the cobalt 387.1-mμ line was sufficiently narrow to enable both lines to be completely resolved from each other when cobalt was added to the sample solution as an internal standard. The cobalt 387.1-mμ line is shown as the dashed curve in Figure 1. Cowan and Dieke (*3*) have pointed out that slit widths of 0.030 mm. or larger should be used in cases where there is a possibility of self-absorption. However, no perceptible self-absorption was noticed for iron for concentrations up to 600 p.p.m.

CALIBRATION CURVES

***L* minus *H* Method.** Standards were prepared by appropriate dilution of the standard stock solution of iron with demineralized water. The hydrochloric acid concentration in these solutions was adjusted such that, on final dilution, the concentration would be approximately 0.3*M*. The luminosity of the background, *H*, at a point near the base of the analysis line, was subtracted from the total luminosity reading of the individual standards, *L*, at the line peak (386.0 mμ) to give net relative luminosities. The background reading should be taken at the base of the 386.0-mμ iron line on the long wave-length side. This wave length minimizes the overlap of any magnesium band remnants and also serves as a suitable background point for the cobalt 387.1-mμ line when an internal standard is added to the sample. The net relative luminosities were plotted against the concentration of iron in the standard solutions to give the calibration curve, Figure 2.

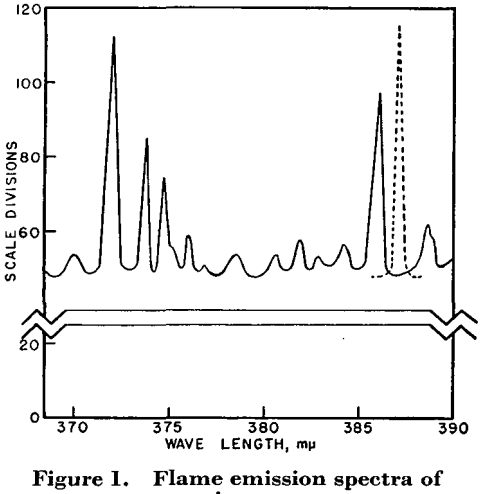

Figure 1. Flame emission spectra of iron

– – – – – Cobalt 387.1-mμ line for 400 p.p.m. present Concentration, 100 p.p.m. iron and 0.050-mm. slit width

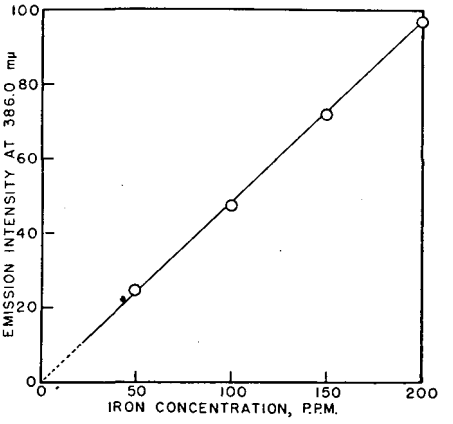

Figure 2. Calibration curve, *L* minus *H* method

Examination of the calibration curve discloses the fact that the straight-line relationship could be extrapolated through the zero point. Theoretically, then, very low concentrations of iron could be determined if excitation conditions could be adjusted accordingly, or if sufficient instrumental amplification were available. However, the experimental results for amounts of iron less than 25 p.p.m. were not reproducible sufficiently for accurate quantitative work. Consequently, the recommended sample, or aliquot portion thereof, should be chosen so that it contains at least 25 p.p.m. of iron. The instrument was calibrated by a series of standards run before and after analysis.

Internal Standard Method. The second type of calibration curve was obtained by the method of internal standardization as proposed by Cholak and Hubbard (*2*). In this method intensity ratios are measured—that is, the ratio of the net relative luminosity of the analysis line to that of an element added to the sample in constant amount, the latter being the internal standard. Such a pair of lines are referred to as an analysis pair. The advantage in using an internal standard is that it affords compensation for many factors, most of which are very difficult to control in practice. These include variations in flame temperature, fluctuations in fuel or oxygen pressure, and interference from other elements.

Ahrens (*1*) has listed several factors which must be considered when choosing an internal standard:

It should be an element which has a negligibly small concentration in the analysis specimen.
Internal standard and analysis lines should have similar excitation potentials.
The internal standard line should be free from self-absorption.
Analysis and internal standard lines should be roughly the same wave length.

The cobalt 387.1-mμ line satisfies all of these conditions and was used in this investigation. No self-absorption was noticed

for either the iron 386.0-mμ line or the cobalt 387.1-mμ line. This may have resulted from use of slit widths of the order suggested by Cowan and Dieke (*3*) for minimizing this trouble.

The calibration curve was obtained by the analysis of a series of standard iron solutions, each containing a fixed amount of cobalt. The appropriate background readings were subtracted from the individual iron 386.0-mμ and cobalt 387.1-mμ luminosities to obtain net relative luminosities. Then, the ratio of the net relative luminosity of the iron line to that of the cobalt line was plotted versus the respective iron concentration on log-log graph paper. A straight-line calibration curve results. Working curves showed a slight irregular drift which made it necessary to prepare a fresh calibration curve for each series of samples.

Influence of Acids (including anions). The effect of various anions was determined, particularly those which might be introduced during sample dissolution. A series of several concentrations of each acid, or of the anions as their ammonium salts, was prepared with 100 p.p.m. of iron present in each of the samples. The results are tabulated in Table II. Acetate and perchlorate ions were found to be particularly detrimental in the determination of iron. The results obtained for hydrochloric acid are in agreement with the spectrographic results reported by Ells (*4*)

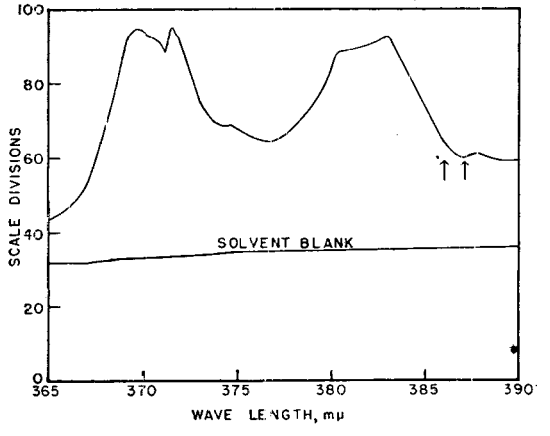

Figure 3. Flame band spectra of magnesium

Arrows indicate peak wave lengths of iron 386.0-mμ and cobalt 387.1-mμ lines
Concentration, 500 p.p.m. magnesium and 0.050-mm. slit width

Table II. Effect of Anions as Their Acids on Flame Photometric Determination of Iron[a]

Anion Added	Concn., *N*	Iron Found, P.P.M. at 386.0 mμ
Acetate[b]	1.0	103
	2.0	109
	3.0	108
Chloride	1.0	100
	2.0	99
	3.0	99
Fluoride	50[c]	99
	100	101
	500	101
Nitrate	1.0	100
	2.0	101
	3.0	100
Perchlorate	1.0	98
	2.0	96
	3.0	92
Phosphate	100[c]	104
	500	98
	1000	97
	5000	101
Sulfate[b]	1.0	100
	2.0	101
	3.0	96

[a] 100 p.p.m. of iron present in all cases.
[b] Values obtained by the internal standard method.
[c] Concentration in p.p.m.

using a spray chamber type atomizer. Ells found no interference up to 2*M* solutions of the acid but an error of 4% for 3*M* hydrochloric acid solutions. When acids must be introduced, nitric or hydrochloric are recommended so long as their maximum concentration does not exceed 2.5*M*.

Influence of Other Elements. A major part of the experimental work was concerned with determining radiation interferences caused by the various elements normally associated with iron in alumina refractories, glasses, and limestone. For each element tested a series of solutions was prepared containing several known concentrations of the suspected interference and generally 100 p.p.m. of iron. Readings were taken at the peak of the iron line, 386.0 mμ, at the background minimum between the iron and cobalt peaks, 386.5 mμ, and at the cobalt line peak, 387.1 mμ. The amount of iron found in each sample was then calculated by two methods: the *L* minus *H* method and the internal standard method. Analyses were not repeated for the internal standard method when the *L* minus *H* method proved satisfactory. Table III gives the results obtained by each method.

From these studies the elements can be grouped into five categories:

1. Elements which offer no interference. These include boron, sodium, titanium, vanadium, and the ammonium ion.
2. Elements exhibiting general background radiation. This occurs with many of the elements when present in relatively high concentrations. This type of interference was compensated in both methods of measurement employed.
3. Coincidences, or near coincidences, such as the overlap of the magnesium band system centered about 383 mμ with the iron 386-mμ line, as shown in Figure 3. Manganese exhibits a similar type of interference owing to its band spectra.
4. Interference with combustion processes was observed for aluminum, lithium, and zinc. However, through use of the internal standard procedure, good results were obtained for iron in the presence of these elements.
5. General interference at either the background or the peak of the iron or cobalt lines is observed for calcium, barium, and potassium when these elements are present in amounts exceeding 50 p.p.m.

METHOD OF PROCESSING SAMPLES

For siliceous minerals and glasses, weigh samples containing 0.9 to 7.1 mg. of ferric oxide into platinum crucibles. Moisten with demineralized water, then add 10 ml. of 48% hydrofluoric acid and 0.5 ml. of 36*N* sulfuric acid. Heat to fumes of sulfuric acid. Cool and then add an additional 5 ml. of hydrofluoric acid and evaporate to dryness. Ignite carefully until fumes of sulfuric acid cease to be evolved. Cool and add 10 ml. of 6*N* hydrochloric acid. Warm until the sample dissolves. If any residue remains, repeat this treatment.

Transfer the entire sample to a 25-ml. volumetric flask. Add 5.00 ml. of the standard cobalt solution and dilute to the mark with demineralized water. Aspirate the samples and measure the luminosities at 386.0, 386.5, and 387.1 mμ. Read the amount of iron present from the calibration curve.

When considerable amounts of magnesium, calcium, barium, or potassium are either known or suspected to be present in the sample, generally amounts exceeding 50 parts per 1000 parts of sample, a preliminary separation of iron is necessary. To the solution obtained following the dissolution of the sample, add a few drops of 30% hydrogen peroxide, and then add a considerable excess of filtered, concentrated ammonia. Stir the solution thoroughly and heat to boiling. Filter off the hydrous ferric oxide and wash it with hot water. Dissolve it in 10 ml. of 1*N* hydrochloric acid and wash the filter with demineralized water. Collect the filtrate and washings in a 25-ml. volumetric flask and proceed as described.

DISCUSSION

Among the usual constituents of siliceous materials several elements offer serious interference to the flame spectrophotometric determination of iron. Large amounts of all the alkaline earths and the alkalies, with the exception of sodium, enhance the flame emission of iron. The cause perhaps is the altering of temperature of the oxyacetylene flame by these elements. It has been shown (*5*) that the population of an electronic state of low energy is determined mainly by thermal processes around a flame temperature of 2000° K. Most of the elements exhibit their full

Table III. Influence of Cations on Flame Photometric Determination of Iron

(100 p.p.m. of iron present in all cases)

Cation Tested	Concn., P.P.M.	Iron Found, P.P.M. $L - H$ method	Iron Found, P.P.M. Internal standard method
Aluminum	100	104	101
	500	99	102
	1000	98	102
	5000	84	97
Ammonium	100	100	..
	500	100	..
	1000	100	..
	5000	100	..
Barium	100	138	93
	500	141	93
	1000	122	96
	5000	90	100
Boron	100	100	..
	500	101	..
	1000	103	..
	2000	112	..
Calcium	100	96	97
	500	106	91
	1000	108	89
	5000	104	88
Lithium	100	116	100
	500	105	99
	1000	85	92
Magnesium	100	93	..
	500	110	..
Manganese	100	104	..
	300	112	..
Potassium	100	118	92
	500	116	90
	1000	98	..
	5000	102	..
Sodium	500	102	..
	1000	106	..
	5000	103	..
Strontium	100	101	..
	500	105	..
	1000	105	..
	5000	off scale	..
Vanadium	50	99	..
	100	103	..
	500	101	..
Zinc	100	92	99
	500	92	100
	1000	88	99

Table IV. Analysis of Bureau of Standards Samples

Samples Compn.	No. analyzed	Iron with Associated Standard Deviation[a] Certified %	Found with Associated Standard Deviation %
Argillaceous limestone 1a 29 Ca, 7 Si, 2 Al, 1 Mg	7	1.14[b]	1.18 ± 0.04
Borosilicate glass 93 38 Si, 4 B, 3 Na, 1 Al	6	0.053 ± 0.003	0.052 ± 0.001
Burnt refractory 76 26 Si, 20 Al, 1 Ti, 0.5 Mg	6	1.66 ± 0.06	1.76 ± 0.06
Burnt refractory 78 37 Al, 10 Si, 3 K, 2 Ti, 0.5 Mg	5	0.55 ± 0.08	0.60 ± 0.04
Silica brick 102 44 Si, 1 Al, 1 Ca	5	0.46 ± 0.03	0.48 ± 0.02

[a] Standard deviation = $\pm\sqrt{\frac{\Sigma(\pm D)^2}{n-1}}$ where D = deviation from mean.

[b] Certificate of analysis missing.

complement of arc emission lines in the inner cone of an oxyacetylene flame wherein the flame temperature is much higher. This would certainly be true for the readily excited elements among the alkalies and alkaline earths. The maximum temperature for an oxyacetylene flame is not attained immediately above the inner cone, but several millimeters above the tip of the inner cone. Thus, the persistence of free atoms in a highly excited state is probably the cause of the delay in reaching the normal temperature characteristic of the flame mantle surrounding the inner cone. Combustion processes proceed mainly by bimolecular reactions, but the recombination of free atoms is thought to require three-body collisions (*10*), and would therefore be less rapid. The normal flame temperature of the outer mantle cannot be attained until after the completion of the large heat release due to the recombination of free atoms formed in the reaction zone of the inner cone. If the recombination occurs with certain atoms—for example, calcium—then it might result in a form of chemiluminescence and result in a higher temperature in the outer mantle and consequently an enhancement of the iron luminescence.

The internal standard method circumvents this type of interference and enables iron to be determined in the presence of the normal amounts of the alkalies and alkaline earths as would be encountered in alumina refractories and clays, and glasses. Seldom do these elements exceed 30 to 100 parts per 1000 parts of sample.

Aluminum is also a serious interference and does not exhibit any spectral lines or bands of its own in the vicinity of the iron and cobalt lines. When it is present in an oxyacetylene flame, aluminum diminishes the intensity of iron and cobalt lines. The decrease in the intensity of the iron and cobalt spectral lines, as a result of the introduction of aluminum into the flame, is presumably due to the absorption of part of the energy by the aluminum atoms, similar to the effect of aluminum on the flame spectra of the alkaline earths (*13*). However, the attenuation affects the two lines equally, so that satisfactory results are obtained for iron in the presence of large amounts of aluminum when the internal standard method is employed.

Amounts of magnesium in excess of 50 p.p.m. proved to be a serious interference regardless of which measurement method was employed. The cause is the overlap of the vibrational bands on the long wave-length side of the magnesium 383-mμ band system with the base of the iron 386.0-mμ line. The overlap is more pronounced for the nearer iron line than for the internal standard cobalt line which precludes use of the internal standard principle. When magnesium is encountered in samples, it is necessary to carry out a preliminary separation of iron as the hydrous oxide or to incorporate an equivalent amount of magnesium in the standard samples.

Of particular interest is the fact that phosphorus offers no interference, at least in concentrations up to 5000 p.p.m.

Table IV summarizes the results obtained on Bureau of Standards samples. The reproducibility of the flame photometric results was very good. The standard deviation from the mean of replicate samples was approximately 3%.

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The Chemistry of Thorium

Spectrophotometric Determination of Thorium as the Naphthazarin Complex

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Naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) gives with thorium ion purple solutions containing a complex cation in which naphthazarin and thorium have a two to one ratio. The color is detectable at concentrations as low as 0.001 mg. of thorium per milliliter. Such solutions absorb strongly at 5700 and 6185 A., with plateaus in the absorption curves at 5375 A. The complex is optically stable for at least several hours, and its solutions adhere closely to Beer's law in the concentration range 10^{-5} to 10^{-6} mole of thorium ion per liter. Pure thorium salt solutions are conveniently analyzed spectrophotometrically in this concentration range using naphthazarin. Inasmuch as the rare earth metal ions, zirconyl, titanyl, and uranyl, all give color systems with similar absorption characteristics, determination of thorium in samples containing these species is possible only after separation of the thorium.

THE paucity of reactions which are characteristic of the thorium ion but not markedly similar to those of other metal ions (especially the rare earth metal ions) with which thorium is associated in nature is both a distinguishing feature of thorium chemistry and a complicating factor in the analytical determination of that element (*11*). This is particularly true of systems based upon absorption spectra measurements. The thorium ion is colorless, showing no light absorption in the range 2000 to 10000 A., and only a limited number of organic reagents have been described for spectrophotometric determinations (*11*). Perhaps the most successful of these is 2-(2-hydroxy-3,6-disulfo-1-naphthylazo)-benzenearsonic acid (thorin), first proposed by Kuznetsov (*8*) and subsequently employed to great advantage for the microdetermination of thorium (*1, 2, 4, 5, 7, 12*). Any potentially useful colorimetric reagent is of general interest to this problem.

Formánek (*3*) discussed the absorption spectra of the violet solutions produced by addition of the dye alkannet to thorium and rare earth metal salt solutions. Absorption bands for the thorium-containing solutions in the regions 5195 to 5222, 5599 to 5625, and 6055 to 6084 A. were recorded, but no distinctions were noted between the thorium and rare earth metal systems. The recent development of a spectrophotometric method for beryllium based upon alkannin or upon its unsubstituted parent, naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) (*14*), suggests the value of a re-examination and extension of Formánek's work. Accordingly, the reaction between thorium ion and naphthazarin has been studied spectrophotometrically. The nature and composition of the colored complex have been determined, and a sensitive colorimetric method applicable to thorium materials free from rare earths has been developed.

MATERIALS AND APPARATUS

Chemicals. Naphthazarin was prepared and purified by the method of Toribara and Underwood (*13*). The source of thorium ion was a sample of thorium nitrate tetrahydrate (Lindsay Chemical Co.), shown to be free from the rare earth elements. Rare earth metal salt solutions were prepared from mixed or purified (98% to atomic weight quality) oxides from the University of Illinois stocks. All other chemicals were of reagent quality.

Absorption Spectra. All absorption spectra measurements were made with a Beckman Model DU quartz spectrophotometer or with a Cary recording spectrophotometer, using 1.00-cm. quartz cells with the former and 1.00-cm. demountable cells with quartz windows with the latter.

Ion Migration. Ion migrations were observed in a two-compartment glass cell with a separating sintered-glass disk and platinized platinum electrodes, using about 0.1 ampere of current.

SPECTROPHOTOMETRIC STUDIES ON THORIUM-NAPHTHAZARIN SYSTEMS

Absorption Spectra. Anhydrous ethyl alcohol was found to be an excellent solvent for naphthazarin and medium for the thorium ion–naphthazarin reaction. Both thorium nitrate and

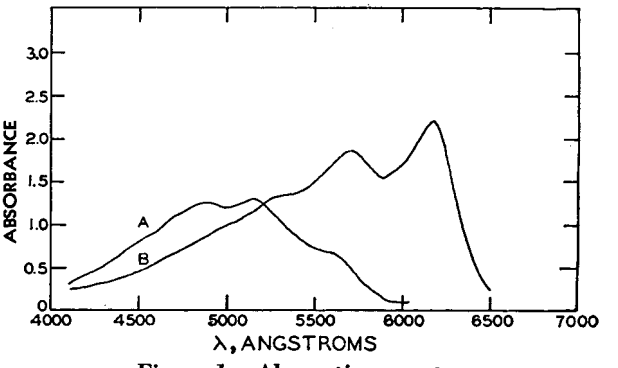

Figure 1. Absorption spectra

A. 2.94 × 10^{-4} mole of naphthazarin per liter of ethyl alcohol
B. Thorium-naphthazarin complex with 1.26 × 10^{-4} mole of thorium per liter

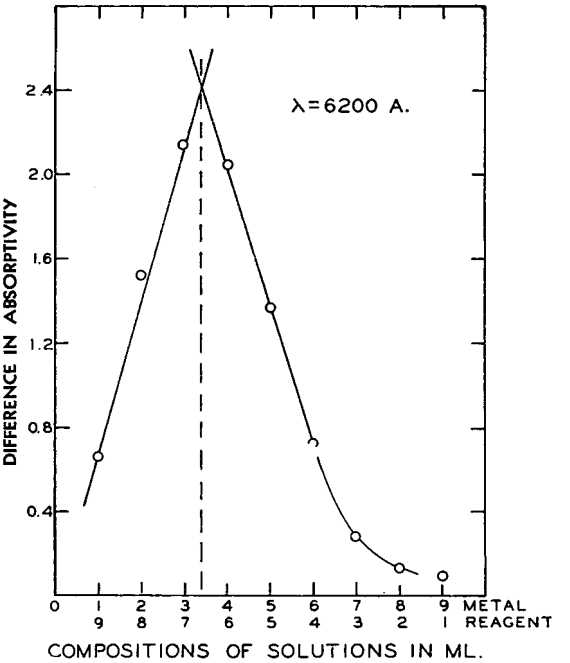

Figure 2. Continuous variations study on thorium-naphthazarin system

Table I. Adherence of Thorium-Naphthazarin Solutions to Beer's Law[a]

Thorium Taken, Mole/Liter ($\times 10^6$)	5700 A.		6100 A.		6200 A.	
	a[b]	ϵ[b] ($\times 10^{-2}$)	a	ϵ ($\times 10^{-2}$)	a	ϵ ($\times 10^{-2}$)
7.0	62.7	146	72.0	167	91.6	213
13.9	63.4	148	73.0	171	94.7	222
20.8	62.3	146	71.7	168	94.7	222
27.8	63.0	147	73.5	172	96.5	226
34.7	63.4	148	74.9	175	96.5	226
41.7	62.5	146	74.5	174	96.4	225
48.5	63.4	148	74.7	175	97.6	229
55.5	63.5	148	75.4	176	97.0	227
Av.	63.0	147	73.7	172	95.6	224

[a] Mole ratio of naphthazarin to thorium = 2 to 1.
[b] Absorptivity, a, is given by relationship $a = (\log_{10} I_0/I)/c\,l$; l is in centimeters and c is expressed as grams of thorium per liter. When c is in moles of thorium per liter, a becomes ϵ, the molar absorptivity.

Table II. Analysis of Pure Thorium Nitrate Solutions

Wave Length, A.	Naphthazarin to Thorium Mole Ratio	Thorium, Mole/Liter ($\times 10^5$) Taken	Found
5700	2:1	0.7	0.695
5700	2:1	1.4	1.4
5700	2:1	3.5	3.5
5700	3:1	4.2	4.15
5700	3:1	8.4	8.25
5700	3.5:1	12.6	12.4
5700	3.5:1	12.6	12.6
6100	2:1	0.7	0.682
6100	2:1	1.4	1.38
6100	2:1	2.1	2.04
6100	2:1	2.8	2.78
6200	2:1	3.45	3.46
6200	2:1	3.78	3.88
6200	2:1	4.13	4.19
6200	2:1	4.82	4.72
6200	2:1	5.86	6.1
6200	2:1	6.2	6.1
6200	2:1	6.89	6.9
6200	2.3:1	6.3	6.1
6200	4:1	4.2	3.9

Table III. Spectrophotometric Studies on Cerium Earth–Thorium Solutions Containing Naphthazarin

Thorium Taken, Mole/Liter ($\times 10^6$)	Cerium Earths Taken, Mole/Liter[a] ($\times 10^6$)	Absorbance at 6200 A. Observed for mixture	Corrected for cerium earth absorption	Observed for pure thorium
7.0	41.7	0.285	0.083	0.149
13.9	41.7	0.410	0.206	0.308
20.8	41.7	0.550	0.348	0.462
27.8	41.7	0.685	0.483	0.628
34.7	41.7	0.850	0.648	0.785
41.7	41.7	0.997	0.795	0.940
48.5	41.7	1.140	0.938	1.110
55.5	41.7	1.280	1.078	1.260

[a] Based upon average atomic weight = 141.

naphthazarin were conveniently used as 0.0021M solutions. The absorption spectrum of the red solution of naphthazarin in absolute ethyl alcohol, as shown in Figure 1, is characterized by absorption maxima at 4875 and 5166 A., with an inflection at 5500 A. Addition of aqueous thorium nitrate solution gives a color change to violet or purple which is visually detectable with at least 0.001 mg. of thorium ion per milliliter. Under comparable conditions, rare earth metal ions give reddish colors of lesser intensities. The absorption spectrum of the thorium-naphthazarin system (Figure 1) has absorption peaks at 5700 and 6185 A., with an inflection at 5375 A. Differences between this spectrum and that of the reagent are sufficient to permit development of a colorimetric procedure.

Composition of Complex. Inasmuch as the absorption spectra of approximately 10^{-5} M solutions with mole ratios of naphthazarin to thorium of 1 to 3, 1 to 2, 1 to 1, 2 to 1, and 3 to 1 were exactly similar except for absorption intensities, the presence of but a single absorbing species was indicated. The absorbing complex was shown to have a naphthazarin to thorium mole ratio of 2 to 1 by an adaptation (*15*) of Job's method of continuous variations (*6*), using varying volumes of 0.0021M solutions in a total volume of 50 ml. of ethyl alcohol and wave lengths in the 5500- to 6200-A. range, as shown in Figure 2. In agreement with valency relationships, this species was shown to be cationic by ion migration. It is not extractable into benzene, chloroform, carbon tetrachloride, diethyl ether, methylcyclohexane, or high boiling petroleum ether, in agreement with an ionic formulation. As it does not pass through collodion or Visking membranes and is flocculated by electrolytes, the complex also possesses colloidal character. A polymeric species involving chelation through bridging naphthazarin groups seems likely.

Stability of Complex. No changes in absorbances were noted for several hours after preparation of the thorium-naphthazarin solutions; thus the system is sufficiently stable for quantitative determinations. Over a 6-week period under laboratory conditions of light and temperature, the absorbance of a $1.68 \times 10^{-4}M$ solution decreased from 1.69 to 1.44 at 5700 A. Subsequent exposure to bright sunlight for 3 weeks caused a further decrease to 0.75. Application of a technique outlined by Martell and Calvin (*10*) to two sets of data gave values of 1.1×10^8 and 9.6×10^8 for the stability constant with respect to thorium ion and naphthazarin, but such data are rendered inaccurate by the colloidal nature of the species.

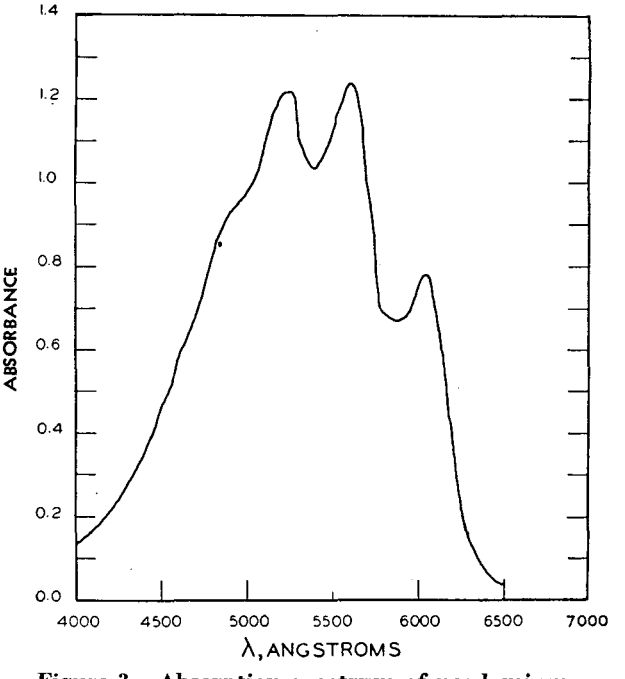

Figure 3. Absorption spectrum of neodymium-naphthazarin complex in ethyl alcohol

10.5 $\times$ 10^{-5} mole of neodymium per liter

Adherence to Beer's Law. Adherence to Beer's law in the concentration range of approximately 2 to 66 $\times$ 10^{-6} mole of thorium ion per liter at wave lengths of 5700, 6100, and 6200 A. was excellent with solutions with mole ratios of naphthazarin to thorium ion of 2 to 1, 3 to 1, 4 to 1, 5 to 1, and 10 to 1, as shown by typical data for the 2 to 1 system in Table I. Naphthazarin in excess of a 2 to 1 stoichiometry enhances absorption at 5700 A. (Figure 1) but has little effect at 6100 A. in quantities up to 5 to 1. Solutions of mole ratios below 2 to 1 deviate markedly from Beer's law. The larger absorptivities at 6200 A. recommend this wave length.

Analysis of Pure Thorium Salt Solutions. Results given in Table II summarize data obtained by application of the naphtha-

zarin procedure to thorium nitrate solutions of various concentrations.

EFFECTS OF OTHER SPECIES

Rare Earth Metal Ion–Naphthazarin Systems. The absorption spectra of rare earth metal salt solutions (La^{+++}, Pr^{+++}, Nd^{+++}, Sm^{+++}, Gd^{+++}, Er^{+++}, Y^{+++}) treated with enthanolic naphthazarin in 2 to 1 mole ratios are all similar to that given for neodymium in Figure 3, the wave length of maximum absorption varying from 6065 A. for lanthanum to 6010 A. for erbium. That such solutions absorb appreciably at 6200 A. suggests difficulty in attempting thorium determinations in the presence of rare earth species.

Attempted Thorium Determinations in Presence of Rare Earth Metal Ions. Since the individual rare earth metal ions showed essentially identical behaviors with naphthazarin, mixtures amounting to a cerium group combination of average atomic weight about 141 and an yttrium group combination of average atomic weight about 125 were used. Both behaved in essentially the same fashion, with displacement of the thorium absorption peak by about 50 A. and considerable contribution of rare earth absorption to this peak in equimolar thorium–rare earth metal ion systems and disappearance of the thorium peak with intensified rare earth absorption in an approximately 6 to 1 mole ratio system. Detailed studies on systems containing varying ratios of thorium and rare earth metal ions at 6200 A. showed marked variations between thorium found and thorium present, even after correction for absorptions produced by the rare earth systems when taken alone, as shown by the typical data in Table III.

Effects of Other Cationic Species. Uranyl, zirconyl, and titanyl ions all give bluish to purple colors with naphthazarin, with absorption spectra closely comparable with that given by thorium ion.

CONCLUSIONS

The colored complex formed between naphthazarin and thorium ion provides a sensitive method for the detection of the latter and for its determination at concentrations of about 10^{-5} to 10^{-6} mole per liter. In contrast to the thorin procedures, light absorption due to the complex occurs in a spectral region where the reagent does not absorb, obviating correction for reagent absorption. The naphthazarin reaction is not specific for thorium, and the element cannot be determined by this means in admixture with the rare earth elements. However, the success achieved with the mesityl oxide extraction separation of thorium ion from the rare earth metal ions (*1, 2, 4, 5, 9*) in other cases suggests that thorium can be determined conveniently in monazite and other rare earth combinations by the naphthazarin procedure after mesityl oxide extraction.

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Determination of Thorium and of Rare Earth Elements in Cerium Earth Minerals and Ores

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The conventional oxalate method for precipitating thorium and the rare earth elements in acid solution exhibits definite solubilities of these elements. The present work was undertaken to establish conditions overcoming these solubilities and to find optimum conditions for precipitating thorium and the rare earth elements as hydroxides and sebacates. The investigations resulted in a reliable procedure applicable to samples in which the cerium group elements predominate. The oxalate precipitations are made from homogeneous solution at pH 2 by adding a prepared solution of anhydrous oxalic acid in methanol instead of the more expensive crystalline methyl oxalate. Calcium is added as a carrier. Quantitative precipitation of thorium and the rare earth elements is ascertained by further small additions of calcium to the supernatant liquid, until the added calcium precipitates as oxalate within 2 minutes. Calcium is removed by precipitating the hydroxides of thorium and rare earths at room temperature by adding ammonium hydroxide to pH > 10. Thorium is separated as the sebacate at pH 2.5, and the rare earths are precipitated with ammonium sebacate at pH 9. Maximum errors for combined weights of thorium and rare earth oxides on synthetic mixtures are ±0.6 mg. Maximum error for separated thoria is ±0.5 mg.

RENEWED interest by the U. S. Geological Survey in the cerium earth minerals, with the recent discoveries of bastnasite, monazite, and associated minerals in California and Montana, led to studies of chemical and spectrochemical methods (*3, 9*) for determining the rare earth elements and thorium. The procedure developed is applicable to the analysis of ores and

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minerals in which the rare earth elements are predominantly of the cerium group. With minerals in which yttrium is the major constituent, the procedure does not give quantitative recoveries. Although yttrium is not one of the rare earth elements, it must be considered with them because of its similar chemical behavior and its occurrence in all cerium earth minerals.

In the procedure presented, four major features are described:

Double precipitation of thorium and the rare earth oxalates at pH 2, calcium being introduced as a carrier before and after each precipitation. The additions of calcium to the supernatant liquid after the first precipitation indicate complete removal of thorium and the rare earth elements by the appearance of precipitated calcium oxalate within 2 minutes, longer periods of time indicating incomplete removal.

Removal of most of the calcium by precipitating thorium and the rare earth elements at room temperature with ammonium hydroxide at pH > 10.

Separation of thorium from the rare earth elements by a single precipitation of thorium sebacate at about pH 2.5.

Precipitation of the rare earth sebacates in the filtrate with ammonium hydroxide at pH 9.

REAGENTS

Alcoholic Methyl Oxalate Solution. Heat reagent grade oxalic acid dihydrate in an oven at 100° C. for 3 hours. Break up the crust and heat at 100° C. for an additional hour. When cool, dissolve 40 grams of the anhydrous oxalic acid in 100 ml. of reagent grade methanol. Allow the solution to stand at least 3 days. Filter before using; 15 ml. of this solution provide approximately 4 grams of oxalate ion.

Calcium Nitrate Solution. Dissolve 22 grams of C.P. calcium nitrate in 500 ml. of distilled water. Adjust to the green color of bromophenol blue indicator with very dilute nitric acid; 5 ml. are equivalent to 0.05 gram of calcium oxide.

Sodium Hydroxide Solution. Dissolve 200 grams of reagent grade sodium hydroxide in 250 ml. of water. Let stand overnight and decant. Dilute to 400 ml.

Ammonium Hydroxide. Specific gravity, 0.90. Prepare fresh from tank ammonia.

Bromophenol Blue Indicator. Dissolve 0.1 gram of water-soluble bromophenol blue in 100 ml. of water.

Thymol Blue Indicator. Dissolve 0.04 gram of thymol blue in 100 ml. of 60% ethyl alcohol.

Ammonia–Ammonium Nitrate Wash Solution. Dissolve 5 grams of reagent grade ammonium nitrate in 475 ml. of distilled water, and add 25 ml. of ammonium hydroxide, specific gravity of 0.90.

Oxalic Acid Wash Solution. Dissolve 5 grams of reagent grade oxalic acid dihydrate in 500 ml. of distilled water.

Hydroxylamine Hydrochloride. Reagent grade.

Sebacic Acid, C.P.

Ammonium Sebacate Solution. Dissolve 0.75 gram of sebacic acid, C.P., in 18 ml. of distilled water and 1.5 ml. of ammonium hydroxide, specific gravity of 0.90.

Hydrogen Peroxide. Reagent grade, 30%.

All other materials used were of C.P. or reagent grade.

PROCEDURE

Decomposition of Sample. Ignite 0.5 to 2.0 grams of sample in a porcelain crucible at dull red heat to expel moisture. Add 6 to 8 grams of sodium peroxide and fuse at dull red heat for 1 minute. Cool. Transfer the crucible to a 400-ml. beaker and leach the fusion product in 200 ml. of water. Digest the solution on the steam bath for 0.5 hour and allow it to stand overnight at room temperature. Filter the solution through a double filter paper, one 12.5-cm. Whatman No. 42 above one 12.5-cm. Whatman No. 41H filter paper (previously rinsed with 1% sodium carbonate wash solution). Wash 8 to 10 times with 1% sodium carbonate solution. Discard the filtrate (silicon, aluminum, phosphorus, and sodium salts). Wash the precipitate from the papers with a stream of water into the original beaker. Pass through the papers, in small portions at a time, 50 ml. of hot 10% nitric acid containing 5 ml. of 30% hydrogen peroxide. Rinse the papers with water. Heat until dissolution of the precipitate is complete. Remove and thoroughly police and rinse the crucible. Ignite the papers in the crucible, and add the ash to the solution.

Removal of Silica. Evaporate the solution to dryness. Remove the silica in 5% nitric acid containing 1 gram of hydroxylamine hydrochloride. Volatilize silica with a few milliliters of hydrofluoric acid, and fuse the residue with 1 gram of sodium bisulfate. Dissolve the melt in 50 ml. of water. Bring the solution to boiling, and add 50% sodium hydroxide sufficient to provide 5% excess. Digest on the steam bath for 20 minutes. If no precipitate appears, discard the solution. If a precipitate forms, filter the solution through a small Whatman No. 40 paper, and wash the precipitate 10 times with hot 1% sodium carbonate solution. Transfer the precipitate and paper to the main solution, and adjust the volume to 125 ml.

Precipitations of Thorium and Rare Earth Oxalates with Alcoholic Methyl Oxalate Solution. To the solution add 10 ml. of calcium nitrate solution (equivalent to 0.1 gram of calcium oxide), unless the sample is known to contain calcium as a major constituent. Add 5 to 10 ml. of 30% hydrogen peroxide. Heat the solution on the steam bath, add 4 drops of 0.1% bromophenol blue, then add 50% sodium hydroxide solution dropwise and with vigorous stirring to attain the blue-green color of the indicator. If iron precipitate masks the indicator, adjust to pH 3.8 using suitable pH indicator paper. Add 15 ml. of prepared alcoholic methyl oxalate solution slowly and with stirring. With the beaker uncovered digest the solution for 0.5 hour, maintaining the volume at 125 ml. by replacing water lost by evaporation. Remove the beaker from the steam bath, and adjust to pH 2 with sodium hydroxide solution using suitable pH indicator paper. Allow the solution to stand 1 hour.

Pour the supernatant liquid into another beaker, or if the liquid is not clear, filter about 75 ml. of it through a 9-cm. Whatman No. 42 paper. Reserve the paper for subsequent filtering. Add 5 ml. of calcium nitrate solution. Stir and allow the solution to stand until a precipitate of calcium oxalate forms. Precipitation of the added calcium within 2 minutes indicates that precipitation of rare earth elements and thorium has been essentially completed. Return the solution and calcium oxalate to the beaker to stand 1 hour longer. If the added calcium had precipitated in the supernatant liquid within 2 minutes, add 5 ml. more of calcium nitrate solution directly to the beaker and stir. (Otherwise repeat the transfer of the supernatant liquid or the filtration and the treatment with 5 ml. of calcium nitrate solution until precipitation of calcium oxalate occurs within 2 minutes.) Allow the solution to stand 0.5 hour, and filter it through a 9-cm. Whatman No. 42 paper or the reserved paper. Wash the precipitate 8 to 10 times with 1% oxalic acid. Transfer the paper and precipitate to the original beaker, add 20 ml. of concentrated nitric acid, cover, and destroy the paper and oxalates by gently boiling until a few milliliters remain. Evaporate to dryness on the steam bath. Dissolve the salts in 50 ml. of 5% nitric acid containing 5 ml. of 30% hydrogen peroxide, by digesting the solution on the steam bath until clear. Adjust the volume to 125 ml., heat on the steam bath, and repeat the entire procedure for precipitating the rare earth and thorium oxalates, omitting further addition of calcium nitrate solution except for tests on the supernatant liquid. Destroy the paper and oxalates in the original beaker by boiling with 20 ml. of concentrated nitric acid until a few milliliters remain. Evaporate to dryness on the steam bath. Allow the salts to bake at steam-bath temperature for 0.5 hour.

Separation of Calcium from Thorium and Rare Earth Elements at pH > 10. To the dry nitrates add 1 ml. of 1 to 1 nitric acid, 25 ml. of water, and 0.5 gram of hydroxylamine hydrochloride. Digest on the steam bath until completely dissolved. Cool the solution to room temperature and dilute to 100 to 125 ml. Add concentrated ammonium hydroxide with constant stirring to pH > 10, indicated by the blue color produced by a small drop of solution applied to pHydrion (Micro Lab., Brooklyn, N. Y.) indicator paper. Add 5 ml. of ammonium hydroxide in excess and some paper pulp, and allow the precipitate to stand 1 hour covered at room temperature; stir occasionally. Filter the solution through Whatman No. 40 filter paper, previously rinsed with ammonia–ammonium nitrate wash solution, and wash 6 times with the same solution. Drain the excess ammonia from the precipitate by applying gentle suction. If thorium and the rare earth oxides are to be determined together, omit the separation of thorium from rare earth elements and proceed with the precipitation of rare earth elements plus thorium.

Separation of Thorium from Rare Earth Elements. Transfer the precipitate and paper to the original beaker, add 20 ml. of concentrated nitric acid, and destroy the paper by gentle boiling. Evaporate to dryness. Dissolve the salts in 25 ml. of water containing 1 ml. of concentrated nitric acid. Dilute to 75 ml., and add 2 grams of hydroxylamine hydrochloride and 1 ml. of thymol blue indicator solution. Heat the solution to boiling and add 10% ammonia slowly to obtain the bright orange color of the indicator, and the complete disappearance of all pink in the solution. Slowly and with constant stirring add 50 ml. of a boiling solution containing 0.5 gram of sebacic acid and 2 drops of 5% nitric acid. Add a small amount of paper pulp, and digest the

precipitate on the steam bath for 10 minutes. Filter the solution through Whatman No. 42 paper, and wash the precipitate 15 times with nearly boiling water acidulated with 5% nitric acid to the bright orange of thymol blue. Ignite the precipitate to constant weight at 1000° C. Report as thorium dioxide.

Precipitation of Rare Earth Elements plus Thorium. Transfer the precipitate and paper as described for separation of calcium to the original beaker, and pulp the paper with 6 ml. of 1 to 1 nitric acid. Heat on the steam bath and dilute to 150 ml. Add 1 gram of hydroxylamine hydrochloride and cool to room temperature. If thorium has been previously separated, adjust the volume of the filtrate to 150 ml. and cool to room temperature. Excess sebacic acid crystallizes on cooling. Add concentrated ammonium hydroxide dropwise to pH 9, indicated by pHydrion paper becoming dark green when a small drop of solution is applied. Add 20 ml. of ammonium sebacate solution and, if not already present, a small quantity of paper pulp. Allow the solution to stand 0.5 hour with occasional stirring. Filter the solution through Whatman No. 40 paper, previously rinsed with ammonia–ammonium nitrate solution, and wash the precipitate 8 to 10 times with the same wash solution. Drain excess ammonia by applying gentle suction. Ignite under oxidizing conditions at 1000° C. to constant weight. Report as CeO_2, $(RE)_2O_3$-(+ThO_2).

EXPERIMENTAL DATA

Thorium, cerium, and lanthanum nitrates, C.P. grade, were purified by precipitation as oxalates, converted to nitrates, and dissolved in 5% nitric acid. Yttrium nitrate solutions were prepared from spectrographically pure yttrium oxide and from yttrium group oxides furnished by The George Washington University, Washington, D. C. The solutions were standardized by evaporation and ignition to the oxides.

The results given in Table I illustrate the efficiency of removal of calcium from cerium and lanthanum. Alternatively, a second precipitation with ammonium hydroxide alone at pH > 10 can replace the ammonium sebacate precipitation, starting with a 0.5% nitric acid solution in order to minimize the solubility of lanthanum oxide in excess ammonium nitrate (*2*). Second precipitation as the sebacates is preferred because of the more easily filterable flocculent precipitate formed and because of its apparent insolubility in the presence of considerable ammonium nitrate salts at a lower pH.

Results in Table II show separations of cerium from varying quantities of thorium by a single precipitation with sebacic acid at pH 2.5. The pH adjustment is critical, requiring careful addition of ammonia. Precipitation made from solutions with the indicator color even slightly pink gave low results. Solutions approaching the yellow color of thymol blue gave positive errors of as much as 2 mg. The results in Table II show a maximum error of 0.5 mg. where proper pH adjustment was made. Spectrographic examination of the ignited thoria (thorium dioxide) precipitates showed contamination by cerium to be 0.1% or less. The separation is rapid, requiring less than 0.5 hour, and is dependable if the proper conditions are maintained.

In Table III results are given for rare earth plus thorium oxides in standard solutions starting with the oxalate precipitations of the procedure. In experiments 1 to 4, 0.2 gram of calcium oxide added only prior to the oxalate precipitation failed to effectively act as a carrier except when thorium and the rare earth elements were present in minor quantities. Recovery of milligram quantities (experiment 3) was complete; with larger amounts, losses were 2 to 3 mg. Experiment 5, showing complete loss of small amounts of rare earth elements in the absence of any calcium, illustrates the need for using a scavenger. The results of experiments 6 to 8 indicate that at least a 1 to 1 ratio of cerium plus lanthanum to yttrium plus thorium—i.e., experiment 12—must exist for complete recovery with the procedure, cerium and lanthanum perhaps acting as carriers. The losses were probably due to the high solubility of yttrium oxalate. Experiments 9 to 12 show complete recoveries of large quantities of rare earth elements and thorium by repeated additions of calcium ion.

The data given in Table IV were obtained with the over-all procedure on simulated rare earth and thorium silicates and phosphates. The maximum error for rare earth plus thorium oxides was +0.0006 gram. The quantities of thorium and rare earth elements were selected to represent samples ranging from low grade to high grade ores. Quantities of thorium and rare earth elements less than 0.0037 gram were not considered in the present study, but can be assumed to be quantitatively recovered according to Waring and Mela (*14*). The largest amount taken, 0.4524 gram, is more than will be found in 0.5 gram of any cerium earth mineral. The over-all procedure, although somewhat long, appears to be more reliable than conventional methods employing oxalate and ammonium hydroxide precipitations.

The results in Table V are of thoria and rare earth oxide determinations in unknown simulated ores prepared by another member of the laboratory. The low value for thoria obtained in the first experiment probably illustrates the critical nature of the pH adjustment before precipitation with sebacic acid and is the product of overcaution in not adding sufficient ammonia. The loss is compensated by a commensurate positive error in the value for rare earth oxides.

Comparison of determinations of thoria in five monazite samples by using sebacic acid, by other chemical methods, and by a spectrographic method (*3*) are given in Table VI. Although the proposed sebacic acid method generally seems to give slightly lower values than other chemical methods, all the results are in good agreement.

DISCUSSION

Solubility of thorium, rare earth, and yttrium oxalates (*4, 12*) was confirmed by the authors. Experiments showed consistent

Table I. Separation of Calcium from Cerium and Lanthanum by Ammonium Hydroxide and Ammonium Sebacate Precipitations

CaO = 0.4 gram

CeO_2, La_2O_3, Gram		
Taken	Found	Error
0.2196	0.2197	+0.0001
0.2196	0.2197	+0.0001
0.0026	0.0027	+0.0001

Table II. Separation of Thorium from Cerium with Sebacic Acid at pH 2.5

CeO_2 = 0.2 gram

ThO_2, Gram		
Taken	Found	Error
0.0016	0.0018	+0.0002
0.0312	0.0313	+0.0001
0.0624	0.0622	−0.0002
0.0937	0.0940	+0.0003
0.1248	0.1253	+0.0005
0.1561	0.1561	None

Table III. Determination of Rare Earth plus Thorium Oxides in Solution

(Effect of repeated additions of calcium ion on collection of thorium and rare earth oxalates)

Common elements taken (gram). CaO, 0.1–0.2; Fe_2O_3, 0.1; Al_2O_3, 0.1; MgO, 0.1; MnO, 0.05; P_2O_5, 0.002; TiO_2, 0.005

No.	Taken, Gram: CeO_2, La_2O_3	Taken, Gram: Y_2O_3	Taken, Gram: ThO_2	CaO Additions	RE, Th Oxides Found, Gram	Error, Gram
1	0.2970	0.0096	0.0366	None	0.3411	−0.0021
2	0.0821	0.0096	0.0366	None	0.1255	−0.0028
3	0.0017	0.0010	0.0008	None	0.0037	+0.0002
4	0.2927[a]	0.0049	0.0183	None	0.3129	−0.0030
5	0.0013[a]	0.0010	0.0008	None	None	−0.0031
6	0.0045	0.0096	0.0330	3	0.0437	−0.0034
7	0.0045	0.0096	0.0330	3	0.0410	−0.0061
8	None	0.0096	0.0330	3	0.0382	−0.0044
9	0.2927	0.0048	0.0183	2	0.3154	−0.0004
10	0.4098	0.0096	0.0330	2	0.4527	+0.0003
11	0.1849	0.0096	0.0330	2	0.2275	None
12	0.0471	0.0096	0.0330	2	0.0893	−0.0004

[a] Common elements omitted.

Table IV. Determination of Rare Earth plus Thorium Oxides in Known Simulated Minerals and Ores

0.25 gram of 1:1 $Ca_3(PO_4)_2$-feldspar mixture added to each

Taken, Gram			RE, Th Oxides Found, Gram	Error, Gram
CeO_2, La_2O_3	Y_2O_3	ThO_2		
0.4098	0.0096	0.0330	0.4524	None
0.2927	0.0096	0.0330	0.3359	+0.0006
0.1171	0.0048	0.0165	0.1388	+0.0004
0.0471	0.0048	0.0165	0.0683	−0.0001
0.0017	0.0010	0.0008	0.0037	+0.0002

Table V. Separate Determination of Rare Earth and Thorium Oxides in Unknown Simulated Ores

0.25 gram of 1:1 $Ca_3(PO_4)_2$-feldspar mixture added to each

ThO_2, Gram			RE Oxides, Gram		
Taken	Found	Error	Taken	Found	Error
0.0312	0.0296	−0.0016	0.2337	0.2355	+0.0018
0.0624	0.0626	+0.0002	0.2337	0.2337	None
0.0952	0.0943	−0.0009	0.2418	0.2421	+0.0003
0.0040	0.0040	None	0.2418	0.2414	−0.0004

Table VI. Determination of Thorium Oxide in Monazites

	In Per Cent		
	Chemical		
No.	Sebacic acid	Other methods	Spectrographic
1	4.4	4.5	4.4
2	7.1	7.35	7.8
3	8.1	8.24	8.4
4	16.20	16.10	15.7
5	9.60	9.65	9.55

losses of 2 to 3 mg. where double precipitations were made at pH 2. The losses are eliminated for small initial amounts of thorium and the rare earth elements by addition of calcium as a carrier before precipitation (*1, 14*), but for large amounts there are still losses of 2 to 3 mg. Repeated addition of calcium ion is therefore necessary.

Experiments with repeated additions of calcium ion as a carrier revealed that the rate of precipitation as oxalate of the added calcium depended upon the quantity of cerium (and presumably other rare earth elements) in the solution—the greater the quantity of dissolved cerium, the longer the period of time for the appearance of the calcium oxalate precipitate. In a series of experiments 5 mg. of cerium oxide in solution prevented the formation of calcium oxalate (calcium ion added as calcium nitrate equivalent to 0.05 gram of calcium oxide) for about 4 hours, 3 mg. for 2 hours, and 1 mg. for half an hour; whereas in a solution containing no cerium, the calcium oxalate precipitated within 2 minutes. Thus, the appearance of precipitated calcium oxalate can serve as an indicator of the completeness of precipitation of cerium and other rare earth oxalates.

By decanting the clear supernatant liquid after the previous precipitations, the liquid can be tested for rare earth elements in solution by adding a solution of calcium nitrate. Appearance of the calcium oxalate precipitate within 2 minutes indicates the solution to be essentially free of rare earth elements. Two additions of calcium nitrate at intervals of 1 hour after the oxalate precipitation usually were found to be sufficient to indicate complete precipitation of the rare earth oxalates. The ability of other rare earth elements to act as inhibitors has not been investigated.

A solution of anhydrous oxalic acid dissolved in methanol was used to precipitate thorium and the rare earth oxalates from homogeneous solution. This reagent performs equally as well as the more expensive solid methyl oxalate which was first introduced by Willard and Gordon (*17*) as a precipitant from homogeneous solution. Although the prepared alcoholic solution hydrolyzes faster than the solid reagent, it produces easily filterable crystalline oxalates.

Calcium and sodium salts were successfully removed by precipitating thorium and rare earth hydroxides from solution at room temperature by adding ammonium hydroxide to pH > 10. This precipitation is customarily done at boiling temperature, which results in volatilization of ammonia and consequent lack of control in maintaining the pH needed for complete precipitation. The high temperature also was found to be faulty in promoting the precipitation of calcium as carbonate at the high pH required. Vickery (*13*) states that unpublished investigations showed that with a rare earth to calcium ratio of 3 to 1, at least five precipitations as hydroxide, presumably from hot solution, are necessary to reduce the ratio to 200 to 1. From cold solutions, however, the authors found that 0.4 gram of calcium oxide could be quantitatively separated from 0.2 gram of cerium and lanthanum oxides by two precipitations with carbonate-free ammonium hydroxide at pH > 10. A single precipitation of the hydroxides at room temperature suffices to remove all but small quantities of calcium; subsequent precipitation with ammonium hydroxide at pH > 10 or ammonium sebacate (*15, 16*) at pH 9 removes the remainder.

Quantitative separations of varying amounts of thorium oxide up to 0.1560 gram from 0.2 gram of cerium oxide were obtained by a single precipitation with sebacic acid (*5, 6, 10*) in boiling solution at about pH 2.5. The reagent does not lend itself easily to double precipitations because of its insolubility in acid solution.

The rare earth elements in the cool filtrate of the thorium precipitate are recovered with ammonium hydroxide and ammonium sebacate at pH 9. Thus the sebacate ion can be used as a precipitant for thorium at a low pH and for the rare earth elements at a higher pH. Although other organic reagents for precipitating thorium may act similarly for the rare earth elements, they have not been reported. Some of the more recently investigated organic reagents for precipitating thorium are *m*-cresoxyacetic acid (*11*), cinnamic acid (*8*), and vanillic acid (*7*).

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Polarographic Determination of Dialkyldithiophosphates

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Six sodium dialkyl dithiophosphates salts, ethyl through butyl, are determined polarographically. Curves of current versus concentration are linear up to $10^{-3}M$. The anodic half-wave potentials become increasingly negative with respect to the saturated calomel electrode as the size of the alkyl group increases. Information concerning the electrode reaction was obtained from the relation between the limiting current and the half-wave potentials at various concentrations of the same salt and by separate experiments, wherein products could be recovered, using a mercury macro electrode at the same half-wave potentials found in the polarographic work. The probable electrode reactions are the oxidation of the mercury to the mercurous ion and a reaction of this ion with the dialkyl dithiophosphate ion to produce free mercury and the mercuric salt. A polarographic determination of a dialkyl dithiophosphate was made at a micro platinum anode and again at a platinum macroelectrode, using the same half-wave potential. Bis-(dialkyldithiophosphoryl) disulfides are formed at this anode.

A METHOD for the polarographic determination of dialkyl dithiophosphates by oxidation at the dropping mercury anode is described and possible electrode reactions are considered. These dithiophosphates are used in dilute solutions for ore flotation (*2*, *7*), insecticides (*6*), and oil additives (*1*). The polarographic behavior of the dialkyl dithiophosphates has not previously been described.

EXPERIMENTAL

Apparatus. A Sargent Model XII photographic recording polarograph was used with an H-type cell (*11*) immersed in a constant temperature bath at 25.0° C. The capillary for the dropping mercury electrode had an $m^{2/3}t^{1/6}$ value of 2.061 mg.$^{2/3}$ sec.$^{-1/2}$ at −0.15 volt *vs.* saturated calomel electrode (S.C.E.) in the supporting electrolyte used for the analysis. A Leeds and Northrup Type K potentiometer with a Type R mirror galvanometer served for instrument calibration. For a supplementary experiment designed to give additional information on the nature of the electrode reactions, a stationary platinum microelectrode as described by Müller (*13*) replaced the dropping mercury electrode. The cell *IR* drop was measured with an impedance bridge from the mercury well through the saturated calomel electrode.

Equipment for the controlled potential synthesis of macro quantities of product, at the half-wave potentials observed in the polarographic work, was similar to that described by Lingane (*10*), except that a porous cup was used to separate the two electrode compartments. A similar arrangement was employed, with the indicated change of electrode, for the electrolysis at a macro platinum anode.

Reagents. The free diethyl, di-*n*-propyl, diisopropyl, diisobutyl, di-*n*-butyl, and di-*sec*-butyl dithiophosphoric acids were all freshly prepared starting with the desired alcohol and phosphorus pentasulfide (*3*, *4*, *16*), all of which distilled at 3-mm. pressure (*8*). The di-*n*-butyl and di-*sec*-butyl compounds, however, decomposed excessively; hence their sodium salts were prepared from crude acids.

The free acids were converted to their respective sodium salts by the addition of an equal volume of anhydrous diethyl ether and subsequent treatment with an excess of anhydrous sodium carbonate. The sodium carbonate was added slowly with stirring to avoid excessive frothing. After reaction ceased, the volume was tripled by the further addition of anhydrous acetone. The product was filtered through a large sintered-glass funnel and the filtrate was evaporated at 50-mm. pressure at 45° to 50° C. Large crystallizing dishes were used to provide shallow layers. The solid products were washed with petroleum ether and dried overnight in a vacuum desiccator. These salts should be protected from moisture during preparation or a gummy product will be obtained.

Analyses for sulfur and phosphorus are shown in Table I.

Table I. Analyses of Sodium Dialkyl Dithiophosphates and Disulfides for Phosphorus and Sulfur

Compound Analyzed	S, %		P, %	
	Found	Theory	Found	Theory
Diethyl salt	30.89	30.79	14.75	14.90
Di-*n*-propyl salt	26.52	27.11	13.23	13.11
Diisopropyl salt	26.95	27.11	13.40	13.11
Di-*n*-butyl salt	24.08	24.06	11.77	11.68
Diisobutyl salt	23.72	24.06	11.80	11.68
Di-*sec*-butyl salt	23.78	24.06	11.79	11.68
Disulfide from diisopropyl salt[a]	29.68	30.08	15.01	14.46
Disulfide from diethyl salt[b]	34.73	34.69	16.73	16.75

[a] Bis-(*O,O*-diisopropyl dithiophosphoryl) disulfide.
[b] Bis-(*O,O*-diethyl dithiophosphoryl) disulfide.

Supporting Electrolyte. Perchloric acid, 0.1000*N*, was used as a supporting electrolyte. All solutions were made to contain the same concentration of this reagent.

Compounds Prepared for Comparative Purposes. Certain compounds were prepared for identification of their properties with those of compounds isolated at the macro mercury and platinum anodes.

Bis-(*O,O*-diisopropyl dithiophosphoryl) disulfide was prepared by oxidizing the sodium salt with iodine in potassium iodide solution. Upon recrystallization of the resulting solid from ethyl alcohol the product melted at 93.8° to 94.6° C. Determination by the Cottrell method gave a molecular weight of 418 as compared with 426.58 for the theoretical value. The corresponding ethyl compound was prepared by reaction of sodium diethyl dithiophosphate with copper sulfate, whence the disulfide was extracted from ether and dried. The ethyl bis compound is a nearly nonvolatile oil. The molecular weight was determined as above to be 382 while the theoretical value is 370.46. The analysis calculated for $(C_2H_5O)_2P(S)SS(S)P(OC_2H_5)_2$: carbon, 25.92; hydrogen, 5.44; found carbon, 26.03; hydrogen, 5.54. Analyses for sulfur and phosphorus on the two compounds prepared are shown in Table I.

Mercuric di-*n*-butyl dithiophosphate was prepared (*12*) by reaction of the mercuric ion with the sodium salt and was found to have a melting point of 60.0° to 61.5° C. The *n*-propyl compound was prepared in a similar manner. The melting point was 67.3° to 69.6° C. with indications of decomposition. The same two mercuric dialkyldithiophosphates were made by reacting mercurous nitrate in 0.1*N* perchloric acid with the sodium dialkyl dithiophosphate, the latter in equivalent amounts or in slight excess. In the latter case, the products are gray solids, easily separable by acetone extraction into equivalent amounts of free mercury and the corresponding mercuric dialkyl dithiophosphate. The reaction is: $Hg_2^{++} + {}^{-}SP(S)(OR)_2 \rightarrow Hg + Hg[SP(S)(OR)_2]_2$.

Polarography of Dialkyl Dithiophosphates. An H-cell (*11*) holding 30 ml. of a solution and containing the desired weight of the sodium dialkyl dithiophosphate in 0.1000*N* perchloric acid was deoxygenated by a stream of purified nitrogen and a polarogram made in accordance with the procedure recommended for the unit used (*17*). Voltage settings obtained from the camera ring scale were checked with the Type K potentiometer. The half-wave potentials were determined also for two dialkyl dithiophosphates at $1 \times 10^{-3}M$ using the stationary platinum electrode as described by Müller (*13*) in place of the dropping mercury electrode. Other equipment and conditions were the same as for previous runs.

Electrolysis with Macro-Size Anodes. The electrolysis of dialkyl dithiophosphates using relatively large electrodes (*10*) was

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made to obtain anodic reaction products in quantities large enough for examination. The cathode was, in each instance, smooth platinum, separated from the anode by a porous cup. The anodes were either mercury or smooth platinum. Voltage measurements of both cathode and anode potentials versus the saturated calomel electrode were made potentiometrically, with a gradual increase of the applied voltage until the anode was at the half-wave potential determined polarographically for the concentration of dialkyl dithiophosphate and electrode used. All determinations were made in 0.1*N* perchloric acid. Oxygen was removed with nitrogen as was done in the polarographic determinations. Stirring was performed mechanically. Products were recovered by filtration through fritted-glass crucibles, or, in the case of the bis-(*0,0*-diethyl dithiophosphoryl) disulfide, formed at the platinum anode by extraction of the aqueous solution with ether.

From Figure 1 and Table II, a linear relationship is seen to exist up to 1 millimole per liter for the polarographic determinations (*14*). Only the normal salts are plotted in Figure 1 since the isopropyl salt behaves similarly to the *n*-propyl salt and the *sec*-butyl and the isobutyl salts have nearly the same values as shown for the *n*-butyl salt.

In Figure 2 it is shown that the log i_d *vs.* $E_{1/2}$ is linear for the polarographic determination of sodium diethyl dithiophosphate. Edsberg (*5*) has shown that $E_{1/2} = E' - 0.03 \times 2 \log i_d$ for the oxidation of 2 Hg→Hg_2^{++} or a slope of approximately 0.06. The value of the slope determined in Figure 2 is 0.068. In consideration of the products formed at a mercury macro-anode, of the reaction observed when mercurous ion is added to a solution of a soluble dialkyl dithiophosphate, and of the information obtained from Figure 2, it would appear that the following sequence of reactions would best account for the behavior observed:

$$2Hg \rightarrow Hg_2^{++} + 2e^-$$

$$Hg_2^{++} + {}^-SP(S)(OR)_2 \rightarrow Hg + Hg[SP(S)(OR)_2]_2$$

It does not appear possible that the dropping mercury electrode is behaving like an inert electrode, as a very different result was obtained with the substitution of a flowing junction type of micro platinum electrode made in accordance with the directions of Müller (*13*). The $E_{1/2}$ *vs.* the saturated calomel electrode shifted from −0.062 volt for the dropping mercury electrode to +0.696 volt for the micro platinum electrode when a $10^{-3}M$ solution of sodium diethyl dithiophosphate was electrolyzed. Similar behavior was observed by Kolthoff and Barnum (*9*) for cystein oxidation at a dropping mercury and at a platinum electrode.

Table II. Polarographic Data on Sodium Diethyl Dithiophosphate

Millimoles per Liter	i_d, μa.	$E_{1/2}$ *vs.* S.C.E., Volt[a]	$i_d/cm.^{2/3}t^{1/6}$
0.166	0.73	−0.010	2.12
0.333	1.39	−0.028	2.01
0.666	2.83	−0.052	2.05
0.833	3.36	−0.056	1.95
1.000	4.17	−0.062	1.98
2.000	6.37	−0.075	1.52
4.000	12.38	−0.097	1.51
5.000	14.60	−0.102	1.46

[a] Corrected for cell *IR* drop.

Table III. Polarographic Data on Dibutyl Dithiophosphates

Millimoles per Liter	*sec*-Butyl, i_d, μa.	Isobutyl, i_d, μa.	*n*-Butyl, i_d, μa.
0.167	0.18	...	0.20
0.333	0.60	0.60	0.63
0.500	0.98	0.99	0.90
0.667	1.48	1.54	1.50
0.833	1.93	1.89	1.91
1.000	2.48	2.24	2.25
5.000	4.88	4.47	3.52
10.000	13.56	10.32	8.57

At the platinum macroelectrode an oil was produced when a $10^{-3}M$ sodium diethyl dithiophosphate was electrolyzed at a potential of 0.70 with respect to the saturated calomel electrode. The sodium diisopropyl dithiophosphate was oxidized similarly. A white solid formed which was filtered, washed with water, and

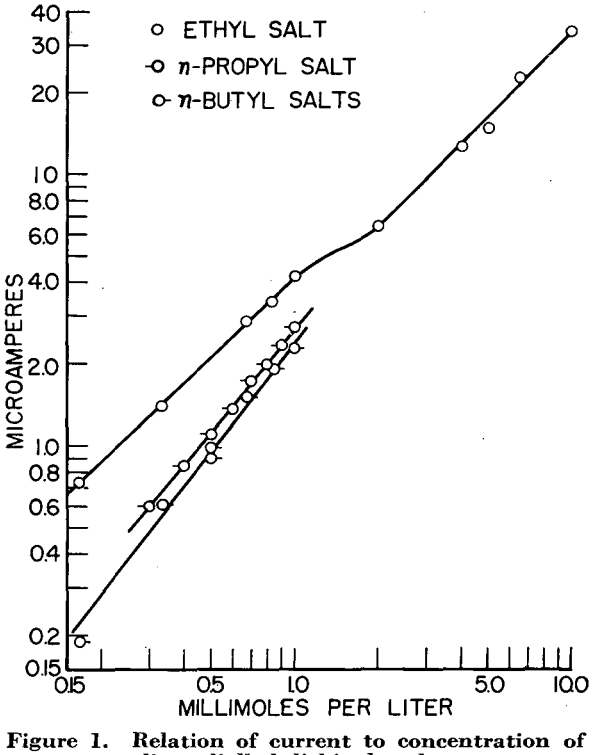

Figure 1. Relation of current to concentration of sodium dialkyl dithiophosphates

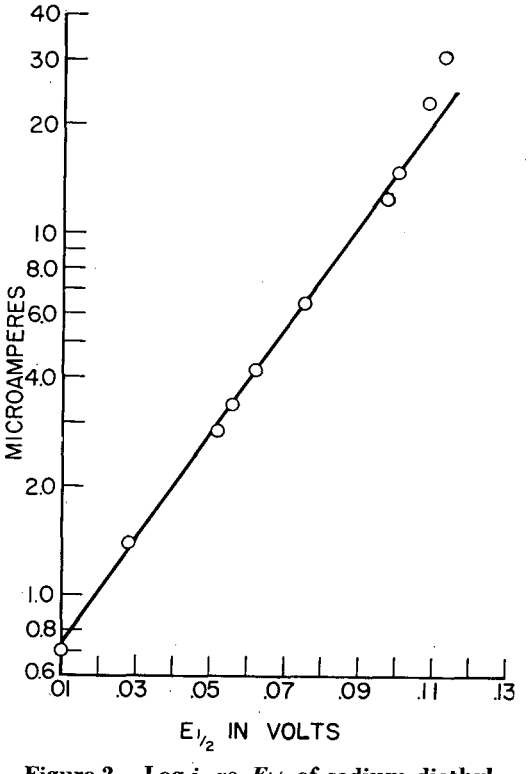

Figure 2. Log i_d *vs.* $E_{1/2}$ of sodium diethyl dithiophosphate

dried over fresh barium oxide. The melting point was 89.0° to 92.0° C. Upon recrystallization from ethyl alcohol the melting point was redetermined as 92.5° to 93.8° C. A mixed melting point with the bis-(*0,0*-diisopropyl dithiophosphoryl) disulfide prepared previously by direct oxidation with iodine produced no change in melting point. It would appear that the reaction at the micro platinum electrode is:

$$2^{-}SP(S)(OR)_2 \rightarrow (RO)_2P(S)SS[S]P(OR)_2 + 2e^{-}$$

The $E_{1/2}$ *vs.* saturated calomel electrode values for the dialkyl dithiophosphates become increasingly negative as the molecular weight increases. At $0.5 \times 10^{-3}M$ the $E_{1/2}$ values determined were for isopropyl, −0.104 volt; *n*-propyl, −0.131 volt; *sec*-butyl, −0.203 volt; isobutyl, −0.200 volt; and *n*-butyl −0.204 volt. This increase would be expected if insoluble precipitates of increasingly lower solubilities were being formed (*15*). At concentrations up to $1 \times 10^{-3}M$ the i_d values for all the butyl salts studies are nearly identical, with deviation becoming apparent only at concentrations above 1×10^{-3}. Results are shown in Table III.

ACKNOWLEDGMENT

The authors wish to express appreciation to Dale McCowen and Gary Babcock for repeating part of the experimental work described.

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Polarographic Measurement of *l*-Noradrenaline and *l*-Adrenaline

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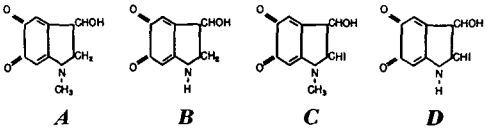
This work was initiated to meet the need for an accurate and simple method for the measurement of *l*-adrenaline and *l*-noradrenaline. The compounds were converted by iodate oxidation to their derivatives iodoadrenochrome or iodonoradrenochrome. Aliquots of the reaction solutions were measured polarographically. Half-wave potentials for iodoadrenochrome and iodonoradrenochrome were found to be $E_{1/2}$ = +0.03 volt and $E_{1/2}$ = +0.02 volt, respectively, in 0.1*M* acetic acid-acetate buffer pH 4.52, 0.01% in gelatin. A linear relation between concentration and diffusion current was found over the investigated range of the equivalent of 1 to 50 γ of adrenaline or noradrenaline. Rapid and accurate routine analysis of these amines can be carried out by the described method.

STUDIES of adrenal medullary activity in man have been handicapped by the lack of an accurate and reasonably simple method for the measurement of adrenaline (epinephrine) and noradrenaline (Arterenol). The bioassay method developed by von Euler (*5*) is carried out on two isolated or intact animal organs. The quantity of adrenaline and noradrenaline in an unknown mixture is calculated from the difference in response of the hen's rectal cecum or rat uterus and the cat's blood pressure, as compared with the response to known amounts of the two amines. The chemical methods now available have not been entirely satisfactory. Early emphasis on the colorimetric measurement of various derivatives of the amines (*1, 5, 13*) has been superseded by more sensitive fluorimetric techniques (*9, 11*). During the course of these studies, the publications of Weil-Malherbe and Bone (*14*) and Manger and others (*12*) have come to the authors' attention. They have not had the opportunity to examine this method. Although the sensitivity has thus been increased, it has not been conclusively established that the fluorescence measured is entirely that of the adrenaline or noradrenaline derivatives.



A *B* *C* *D*

Figure 1. Structure of amines

A. Adrenochrome
B. Noradrenochrome
C. Iodoadrenochrome
D. Iodonoradrenochrome

The present study is concerned with the application of the polarographic method to the measurement of adrenaline and noradrenaline. The catechol nucleus of adrenaline is oxidized at a potential so positive that its measurement at the dropping mercury electrode would not be practical. However, *p*- and *o*-quinones are easily reduced and produce well defined, reversible polarographic waves which bear a linear relationship between concentration and diffusion current. Adrenochrome, the *o*-quinone of adrenaline, although reversibly reduced over a wide range of pH, as shown by Wiesner (*15*), is unstable and eventually precipitates as melanin. Under suitable conditions, adrenaline is converted quantitatively to a stable derivative, iodoadrenochrome (Figure 1). Since this compound has the same *o*-keto structure as adrenochrome, it was expected that this derivative would produce a polarographic wave at a similar potential.

The present study presents a modification of the iodate oxidation described by Bouvet (*3*) for the conversion of adrenaline to iodoadrenochrome. The method has been extended to the prep-

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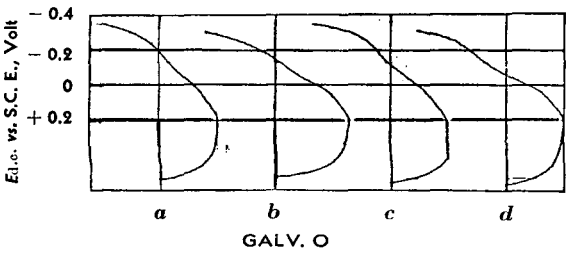

Figure 2. Typical polarographic curves

0.1*M* HAc-Ac buffer, pH 4.52, 0.01% in gelatin

	Iodoadrenochrome		Iodonoradrenochrome	
	a	*b*	*c*	*d*
Concn.	$2.27 \times 10^{-4}M$	$4.52 \times 10^{-4}M$	$3.45 \times 10^{-4}M$	$6.88 \times 10^{-4}M$
T, sec.	3.8	3.8	4.2	3.9
Sens, μA.	5	5	5	5
H (mm).	17.3	17.3	16.8	16.6
D	3	3	3	3

aration of the iodonoradrenochrome. Both compounds can easily be prepared and aliquots of the reaction solutions can be polarographed without purification or separation from excess reagents. Diffusion currents increase linearly with concentration over the range investigated of 1 to 50 γ in 5 ml. of total volume.

PREPARATION OF IODOADRENOCHROME

Approximately 10^{-2} to $10^{-3}M$ stock solutions were prepared from crystalline adrenaline bitartrate or free adrenaline in 2% acetic acid. Two tenths of a milliliter of hydrochloric acid and methanol were added to dissolve the less soluble free adrenaline in the preparation of the $10^{-2}M$ solutions. These stock solutions remained colorless for several days under refrigeration.

To an adrenaline aliquot containing approximately 10^{-3} millimole, 2% acetic acid was added to give a total reaction volume of approximately 5 ml. The exact total volume was found to be not critical if the solution was well buffered. Six millimoles of 0.1*N* potassium iodate per millimole of adrenaline were added and the solutions were mechanically stirred for exactly 4 minutes. During stirring the solution changes from colorless to a final color ranging from salmon pink to red-orange or deep red, depending on the initial concentration of adrenaline present. After stirring exactly 4 minutes, 2*N* sulfuric acid (1 ml.) was added, which decreased the pH to 1. The color of the solution changes to mauve or deep purple, indicating the substitution of an iodine atom in the adrenochrome indole ring. An amount of 0.2% potassium iodide (the stock solution was acidified with 0.5 ml. of 2*N* sulfuric acid) equivalent to 1.7 millimoles per millimole of adrenaline was added as soon as the mauve or purple color of iodoadrenochrome appeared (less than 0.5 minute). Aliquots of this solution were then pipetted into the polarographic cell which contained 0.1 ml. of 0.5% gelatin and sufficient 0.1*M* acetic acid–acetate buffer (of pH 4.52) to give a final total volume of 5 ml. After the addition of the iodoadrenochrome aliquot, oxygen was removed from the buffer solution by bubbling a stream of prepurified nitrogen through the cell for 10 to 15 minutes. Complete removal of oxygen is required since it gives a wave in the vicinity of the amine derivatives. The prepurified gas was passed through two bottles containing Fieser's solution (*3*), through a solution of lead acetate, and finally through distilled water before being admitted to the test solution.

All polarographic curves were recorded on a Leeds and Northrup Electrochemograph, Type E. A 0.1*N* potassium nitrate agar bridge was connected to the reaction vessel with a saturated calomel electrode. The resistance of the external circuit was assumed to be negligible for the small currents recorded. The capillary constant, *K*, was equal to 6.87.

Measurements were made at a sensitivity chosen to give a 5- to 30-mm. diffusion current with anodic-cathodic polarization (±0.2 to −1.0 volt *vs.* S.C.E.). The solutions were rebubbled with nitrogen and a second curve was recorded to confirm the complete absence of oxygen.

Table I. Titration of Excess Iodine with 0.1*N* Thiosulfate

Equivalent of Adrenaline, Mg.	Conversion to Iodoadrenochrome, %
21.4	88
18.2	110
18.2	99
18.2	80
18.2	93
13.65	112
	Av. 97
Equivalent of Noradrenaline, Mg.	**Conversion to Iodonoradrenochrome, %**
33.42	94
33.42	100
33.42	103
	Av. 99

PREPARATION OF IODONORADRENOCHROME

Stock solutions were prepared from crystalline noradrenaline bitartrate monohydrate to contain approximately 10^{-2} to 10^{-3} millimole per milliliter. In contrast to adrenaline, noradrenaline solutions diluted to volume with 0.2*N* sodium acetate were stable for about 4 to 8 hours. Solutions diluted with distilled water showed a color change only after several days. The oxidation was carried out with the same proportions of reagents used for adrenaline, but 0.2*N* sodium acetate was substituted for the 2% acetic acid to give a less acid medium. The oxidation time was increased to exactly 6 minutes. The remainder of the procedure was carried out exactly as for adrenaline. Aliquots were analyzed in the same acetic acid–acetate buffer of pH 4.52.

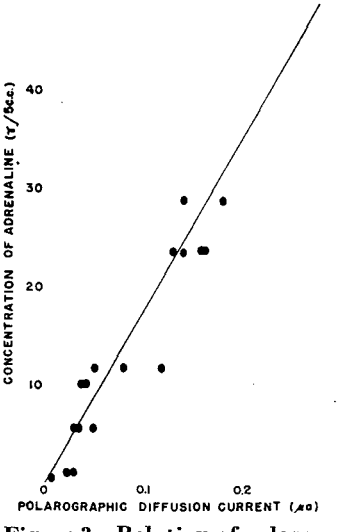

Figure 3. Relation of polarographic diffusion current to the concentration of adrenaline

RESULTS

Aliquots of the iodoadrenochrome or iodonoradrenochrome solutions containing the equivalent of 1 γ or more of adrenaline or noradrenaline produced well defined and reproducible polarographic curves (Figure 2). In 0.1*M* acetic acid–acetate buffer of pH 4.52, 0.01% in gelatin, at 25° C., the observed half-wave potentials were: $E_{1/2}$ = + 0.03 volt for iodoadrenochrome, and $E_{1/2}$ = + 0.02 volt for iodonoradrenochrome.

Standard curves obtained from aliquots of iodoadrenochrome or iodonoradrenochrome solutions containing the equivalent of 1 to 50 γ of adrenaline or noradrenaline (Figures 3 and 4) showed a linear relation between diffusion current and concentration for both compounds. The polarographic data are reproducible for duplicate or varying amounts of amine contained in aliquots of solutions of iodoadrenochrome or iodonoradrenochrome. No difference was observed in conversion of free adrenaline and the bitartrate salt.

To check the conversion of adrenaline and noradrenaline to their iodo derivatives by an independent method, the amines were oxidized following the above procedure and the excess iodine was titrated immediately with 0.1*N* sodium thiosulfate in the presence of 2 ml. of starch solution. Crystals of iodoadrenochrome and iodonoradrenochrome formed in these more concentrated solutions (containing the equivalent of 13 to 33 mg. of amine) did not interfere with the titrations. Calculations were based on the published total uptake of 6 atoms of iodine per molecule of amine following Bouvet (*3*). The titrations of five iodoadreno-

chrome solutions averaged 96.9%; those of three determinations of iodonoradrenochrome solutions, 99.0% (Table I).

The reproducibility of conversion of each amine to its derivative and its subsequent measurement was established by a series of analyses carried out on known amounts of crystalline adrenaline bitartrate and noradrenaline bitartrate monohydrate. Stock solutions were used only on the day of preparation. From two to six aliquots containing the equivalent of 0.158 to 0.738 mg. of adrenaline were converted to the iodo compound according to the procedure given above. In the case of noradrenaline, however, the amounts of each compound were treated as "unknown" —that is, to each solution was added an excess of 0.1*N* potassium iodate (0.4 ml.) and 0.2% potassium iodide (0.8 ml.), sufficient to convert as much as 6.6×10^{-3} millimole (1.1 mg.) of noradrenaline.

Polarographic measurements were made in duplicate on aliquots of the iodoadrenochrome solutions containing the equivalent of about 20 γ of amine. From the diffusion current obtained, the weight of adrenaline or noradrenaline contained in the aliquot was calculated from the slopes of the standard curves (Figures 3 and 4). The total amounts of compound were then found from the known volumes of the reaction solutions. The data are tabulated in Table II. The per cent error of the method is 5 to 10%.

The conversion of either adrenaline or noradrenaline is quantitative and reproducible under rigorously controlled conditions in both concentrated and dilute solutions. The theoretical minimum amounts required for complete conversion of the amines as calculated from Bouvet (*3*) are 6 millimoles of iodate per millimole of adrenaline (or noradrenaline) and 1.7 millimoles of potassium iodide per millimole of adrenaline. Under these conditions, reproducibility of conversion of the amines to their derivatives depends on precise timing of the oxidation reaction and adequate stirring of the solution. Although the wave height is independent of variation in iodate added (Table I), the ratio of iodate to iodide must be kept constant to avoid either a large excess of iodate (greater than about twelvefold) which enhances the iodoadrenochrome wave, or iodide, which itself produces a masking anodic wave.

The applicability of this method to biologic solutions raises certain questions. Adrenaline and noradrenaline cannot be measured individually in the same solution, since the differentiating methyl group, lying in the indole ring, does not significantly affect the half-wave potentials for the respective reactions. In contrast to other methods for the measurement of catechol amines, however, colored or fluorescent substances normally found troublesome do not interfere.

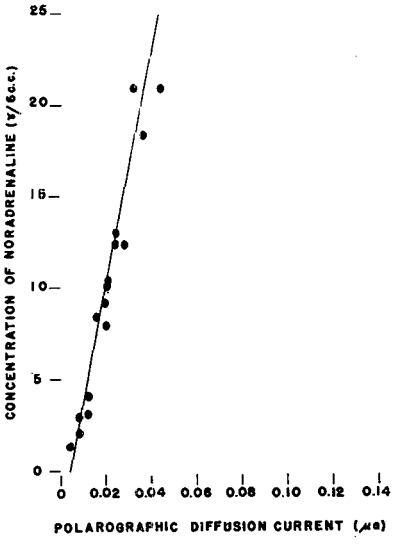

Figure 4. Relation of polarographic diffusion current to the concentration of noradrenaline

Previous workers (*2, 4, 6, 10, 11*) have separated noradrenaline and adrenaline by partition chromatography. Utilizing a paper chromatographic method for the separation of noradrenaline and adrenaline prior to polarographic analysis, preliminary studies in the authors' laboratory indicate the feasibility of collecting eluates containing these amines, converting to the iodo derivatives in the same test tubes and analyzing an aliquot of each. A single conversion and analysis can be done in 20 minutes. Less time is required for a group of samples done in series.

Table II. Accuracy of Measurement of Adrenaline and Noradrenaline after Conversion to Corresponding Iodonor- or Iodoadrenochrome

	Adrenaline		
No. of Conversions (Each Measured in Duplicate)	Polarographic measurement, (av.), mg.	Theoretical, mg.	Error, %
2	0.522	0.499	+ 5
6	0.678	0.738	− 8
2	0.353	0.394	−10
2	0.386	0.362	+ 7
6	0.467	0.527	−11
4	0.333	0.316	+ 5
3	0.148	0.158	− 6
3	0.234	0.246	− 5

		Nonadrenaline		
No. of Conversions (Not Measured in Duplicate)	Millimole KIO_3 Added	Polarographic measurement (av.), mg.	Theoretical, mg.	Error, %
2	0.025	0.487	0.441	+10
1	0.02	0.245	0.220	+11
5	0.008	0.234	0.239	− 2
5	0.02	0.267	0.239	+11
5	0.008	0.252	0.241	+ 5
5	0.02	0.229	0.241	− 5
5	0.009	0.260	0.237	+10
5	0.02	0.240	0.237	+ 1
4	0.01	0.416	0.403	+ 3
4	0.01	0.368	0.398	− 8
7	0.04	0.318	0.365	−13
8	0.01	0.334	0.344	− 3
7	0.04	0.365	0.426	− 4

The polarographic method has the advantage of being rapid, specific, and sensitive. It should be particularly valuable for studies of the output of adrenaline and noradrenaline in urine. Levels of the catechols excreted by normal young males per 24 hours are of the order of 11.5 γ of adrenaline and 29.0 γ of noradrenaline (*7*). This is within the optimum working range of the polarographic method.

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Catalog of Infrared Spectra of 20-Isosapogenin Acetates

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A new series of sapogenins, epimeric with the natural sapogenins at carbon atom 20, was discovered at this laboratory and reported a short while ago. As a means of characterizing these new compounds, the infrared spectra have been obtained. The spectra of 10 of the 20-isosapogenins are presented, as the acetates. The spectra contain much characteristic detail and can be used for ready identification of these compounds.

RECENTLY this laboratory announced the discovery of a new series of sapogenin isomers prepared by treatment of pseudosapogenins with weak acid (*8*). Evidence is presented in another paper (*9*) that these new compounds differ from the naturally occurring sapogenins in having the β configuration of the methyl group attached to carbon atom 20, whereas the natural sapogenins have the 20-α configuration. Infrared spectra of these 20-isosapogenins have been obtained for characterization and are found to contain many strong, sharp absorption bands highly characteristic of the individual compounds. As with the naturally occurring sapogenins (*3*), the 20-isosapogenins have spectra which are more clearly distinguishable in the acetate form than in the hydroxyl form. Hence, in this paper, reference spectra are presented for 20-isosapogenin acetates (Figures 1 to 10). Except for the bands characteristic of hydroxyl and acetate groups, the spectrum of each genin is roughly the same whether in the hydroxyl or acetate form.

EXPERIMENTAL

Preparation and properties of the 20-isosapogenins are described in another paper (*10*), the starting materials being obtained from plants supplied by the Plant Introduction Section, Horticultural Crops Research Branch, U. S. Department of Agriculture, through the courtesy of D. S. Correll. Table I gives some of the physical constants of the 20-isosapogenins. Melting points are meaningless for all but 20-isosarsasapogenin and 20-isosmilagenin, as heat begins to convert the others to pseudosapogenins before the melting point is reached. It is difficult to estimate the purity of a compound which does not have a sharp melting point and which has no literature value with which to compare its optical rotation. Nevertheless, it is felt that these 20-isosapogenin acetates are reasonably pure. Their elemental analyses for carbon and hydrogen are close to theoretical, as given in detail in a companion paper (*10*); their infrared spectra lack bands characteristic of the isomeric pseudosapogenins or natural sapogenins; and their x-ray diffraction patterns look like those of single-phase samples rather than mixtures, with the exception of 20-isodiosgenin acetate which was not examined by x-ray diffraction. This compound had undergone three recrystallizations, however, and should be as pure as the others.

Table I. Physical Constants of 20-Isosapogenins

Compound	Melting Point[a], ° C.		Specific Rotation[b], Degrees	
	Genin	Acetate	Genin	Acetate
20-Isochlorogenin	...	...	−48.6	−39.6
20-Isodiosgenin	...	...	−97.5	−99.0
20-Isogitogenin	...	...	−55.8	−81.9
20-Isohecogenin	...	...	+13.5	+ 7.5
20-Isomanogenin	...	...	+ 1.5	−30.9
20-Isomarkogenin	...	...	+17.7	− 4.8
20-Isosarsasapogenin	176–177	167–168	+31.9[c]	+30.3[c]
20-Isosmilagenin	185	160	−60.3[c]	−48.7[c]
20-Isotigogenin	...	...	−61.8	−64.5
20-Isoyamogenin	...	...	−42.3	−40.2

[a] Kofler melting points.
[b] Rotations at 25° C., 589 mμ, concentrations 8 ± 1 grams per liter, in dioxane except as noted.
[c] Rotations in chloroform.

The infrared spectra were obtained with a Perkin-Elmer Model 21 spectrophotometer using a sodium chloride prism. A "Resolution" setting of 930 was used for the slit program, with a scanning speed of 0.7 cm.$^{-1}$ per second. To accommodate the strong absorption by the solvent between 800 and 900 cm.$^{-1}$, the slit was opened further and the scanning speed was reduced. With a

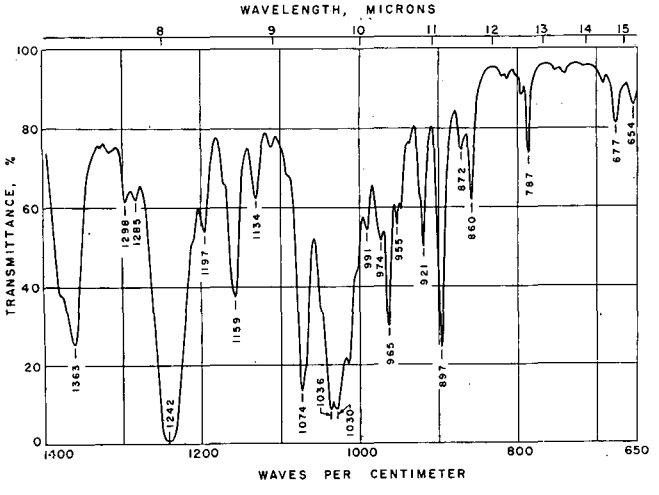

Figure 1. 20-Isochlorogenin diacetate (5α,20β,22ξ, 25D-spirostane-3β,6α-diol 3,6-diacetate)

10.0 grams per liter in CS_2; 1.0-mm. cell

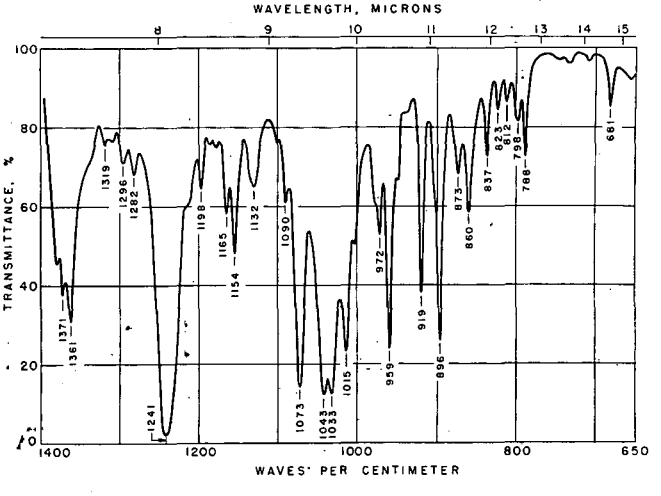

Figure 2. 20-Isodiosgenin acetate (20β,22ξ,25D-spirost-5-en-3β-ol 3-acetate)

10.0 grams per liter in CS_2; 1.0-mm. cell

3-mm. layer of carbon disulfide, even this expedient cannot prevent a distorted curve, as on Figure 8.

DISCUSSION OF SPECTRA

The spectra of the 20-isosapogenins as a class are very different from those of the natural sapogenins as a class. The natural sapogenins are characterized by bands near 860, 900, 920, and 980 cm.$^{-1}$ with a large difference in intensity between the 900- and 920-cm.$^{-1}$ bands (*3*, *7*). On the other hand, this pattern is grossly altered in the 20-isosapogenins, compounds of the "normal" (22b) series having nearly equal 900- and 920-cm.$^{-1}$ bands and compounds of the "iso" (22a) series lacking the 980-cm.$^{-1}$ band. Although the familiar reversal of intensity (*3*, *5*, *7*) of the 900 and 920 bands is lost, there is still a clear distinction in wave number between these two isomeric series, 20-isosapogenins of the normal (22b) series having bands in the ranges 865 to 871, 903 to 905, 917 to 918, 982 to 992, 1080 to 1084 cm.$^{-1}$, while those of the iso (22a) series have bands in the ranges 785 to 788, 860 to 862, 895 to 897, 919 to 922, and 1073 to 1076 cm.$^{-1}$. An occasional interfering band is found, but it is weak and does not disturb the over-all pattern.

Many of the details which characterize the individual sapogenin acetates (*3*) still apply to the 20-isosapogenin acetates. Strength of the acetate bands near 1240 and 1740 cm.$^{-1}$ indicates the number of acetate groups. Complexity of the band near 1240 cm.$^{-1}$ indicates a cis relationship between the C_5 hydrogen and the C_3 acetate (*4*). 12-Keto-20-isosapogenins have the usual carbonyl band (at 1711 cm.$^{-1}$ for both 20-isohecogenin and 20-isomanogenin acetates) and also a strong band at 1065 to 1066 cm.$^{-1}$, which possibly corresponds to the 1075 cm.$^{-1}$ band of the natural 12-ketosapogenins first mentioned by Jones *et al.* (*5*). The Δ^5 unsaturated compounds are recognized by a band at 811 to 812 cm.$^{-1}$ and enhanced intensity of the 836 to 837 cm.$^{-1}$ band.

For individual identification, each of these compounds can be quickly identified by the shape of the curve between 1000 and 1100 cm.$^{-1}$. The identification should, of course, be checked by comparing the complete spectrum of the unknown with that of the chosen reference compound. The two must agree at every absorption band, not only in wave number but also in relative intensity. Figure 4 agrees with the bands listed for the hecogenin isomeride of Callow and James (*1*) and the 750- to 1100-cm.$^{-1}$

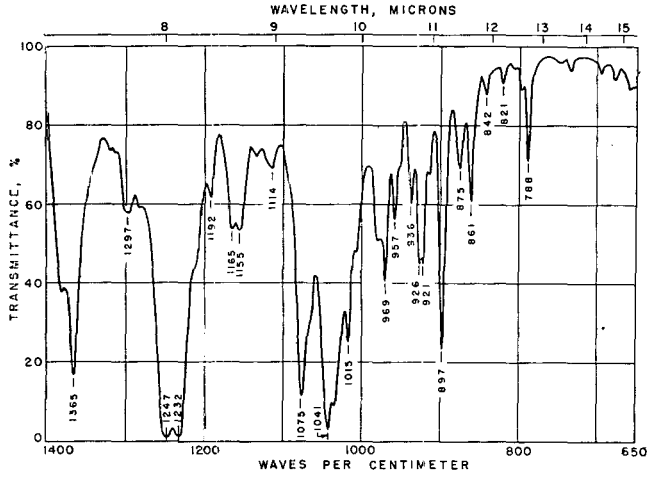

Figure 3. 20-Isogitogenin diacetate (5α,20β,22ξ,25D-spirostane-2α,3β-diol 2,3-diacetate)

10.1 grams per liter in CS_2; 1.0-mm. cell

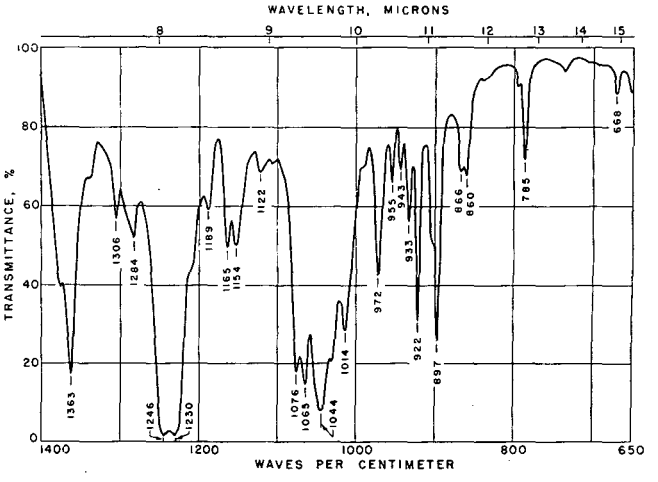

Figure 5. 20-Isomanogenin diacetate (5α,20β,22ξ,25D-spirostane-2α,3β-diol-12-one 2,3-diacetate)

10.0 grams per liter in CS_2; 1.0-mm. cell

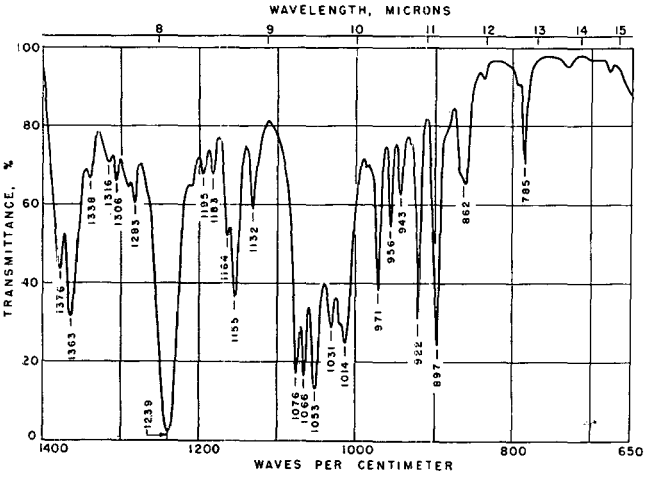

Figure 4. 20-Isohecogenin acetate (5α,20β,22ξ,25D-spirostan-3β-ol-12-one 3-acetate)

10.0 grams per liter in CS_2; 1.0-mm. cell

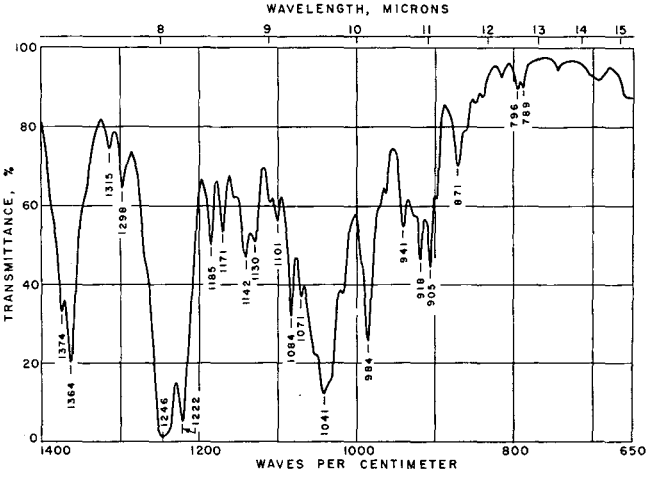

Figure 6. 20-Isomarkogenin diacetate (5β,20β,22ξ,25L-spirostane-2ξ,3β-diol 2,3-diacetate)

10.0 grams per liter in CS_2; 1.0-mm. cell

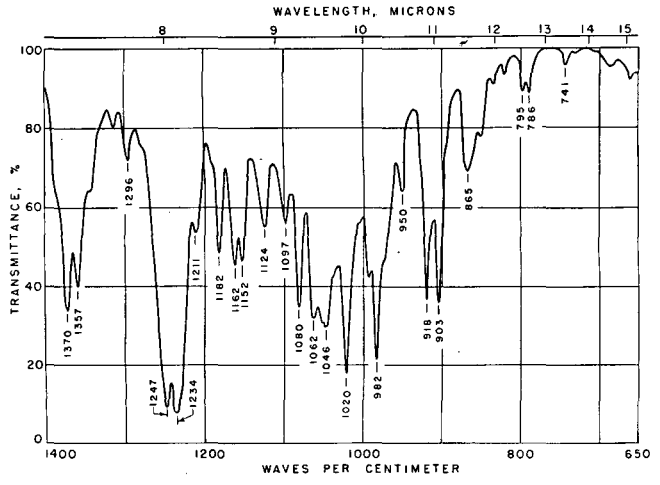

Figure 7. 20-Isosarsasapogenin acetate (5β,20β,22ξ,25L-spirostan-3β-ol 3-acetate)

10.0 grams per liter in CS_2; 1.0-mm. cell

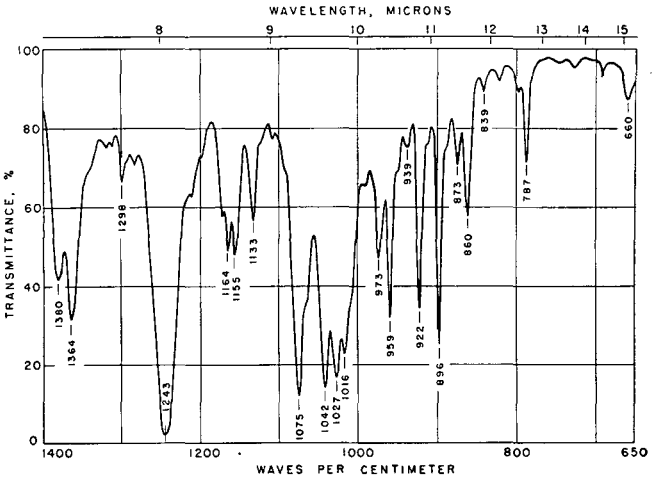

Figure 9. 20-Isotigogenin acetate (5α,20β,22ξ,25D-spirostan-3β-ol 3-acetate)

10.0 grams per liter in CS_2; 1.0-mm. cell

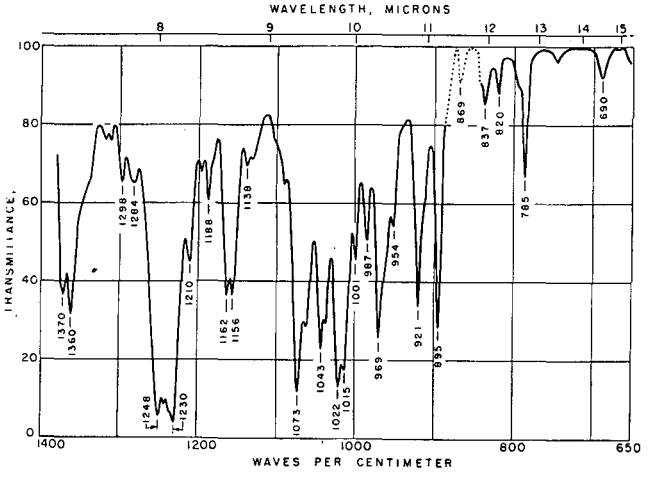

Figure 8. 20-Isosmilagenin acetate (5β,20β,22ξ,25D-spirostan-3β-ol 3-acetate)

4.0 grams per liter in CS_2; 3.1-mm. cell

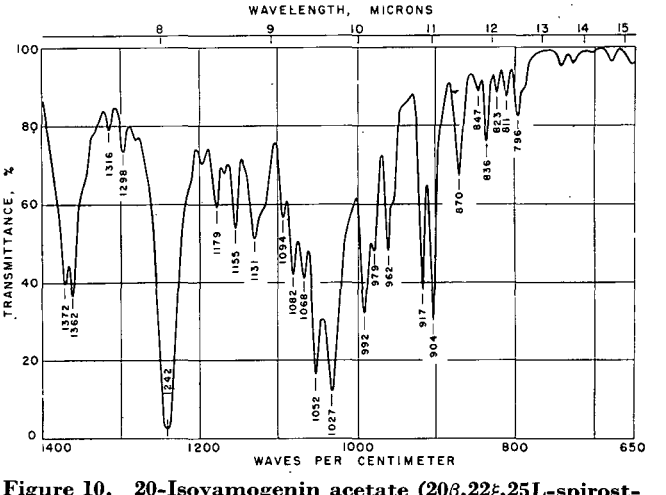

Figure 10. 20-Isoyamogenin acetate (20β,22ξ,25L-spirost-5-en-3β-ol 3-acetate)

10.0 grams per liter in CS_2; 1.0-mm. cell

region of Figure 9 agrees with the anatigogenin acetate curve of Dickson *et al.* (*2*).

ACKNOWLEDGMENT

The authors wish to thank Henry A. Walens, Samuel Serota, Robert F. Mininger, and Monroe E. Wall for supplying the compounds for this study and for measuring their physical properties; and Lee P. Witnauer and Donald Killen for obtaining and interpreting the x-ray diffraction patterns.

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Effect of Temperature on Photometric Determination of Phosphorus in Steel

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Some aspects of the development of the molybdo-vanado-phosphoric acid complex as applied to the photometric determination of phosphorus in steel have been considered. In particular, the claims of several workers with respect to the influence of temperature have been investigated. Of the various methods examined, only one gives results independent of temperature, and the success of this method is attributed solely to the fortuitous formation of sulfate during oxidation of the solution with ammonium persulfate. An improved method for the determination of phosphorus using the more convenient permanganate oxidation is applicable to a wide range of alloy and plain carbon steels. Results independent of temperature and as accurate as those given by the best of other available methods are obtained by the deliberate addition of sulfate to the solution used for dissolving the sample.

THE yellow color formed when excess molybdate and vanadate are added to a weakly acid orthophosphate solution was first investigated by Misson (*8*), and attributed to a molybdo-vanado–phosphoric acid complex. Several photometric methods based on the formation of this complex have been developed for the determination of phosphorus in a wide variety of substances, including steels, iron ores, and biological materials. Work on these methods is still greatly handicapped by an almost complete lack of knowledge of the constitution of the complex.

The effect of temperature on the stability of the complex has been examined by various workers. Murray and Ashley (*9*) found the color stable over the range 20° to 30° C. but not at 10° or at 50° C. Harrison and Fisher (*3, 4*) confirmed this effect, whereas both Center and Willard (*2*) and Hawes (*5*) reported variations with temperature. Kitson and Mellon (*7*) did not report any difficulty, and proposed measuring the color at "room temperature." Hill (*6*), using the method of Kitson and Mellon as applied to the analysis of steel (involving oxidation with ammonium persulfate), showed that results independent of temperature could be obtained only by the use of a blank, and apparently assumed that, by compensating for the iron background color in this way, similar temperature independence would be achieved in other methods including his preferred technique based on a permanganate oxidation.

A method for the determination of phosphorus in steel (unpublished in the literature, but essentially similar to the permanganate method of Hill) was developed in these laboratories, and has been in regular use for a number of years. Experience has shown this method to be markedly sensitive to temperature, and, as laboratory temperatures are not controlled, it was necessary to use a family of calibration curves covering the full temperature range encountered (12° to 32° C.).

Since oxidation with permanganate is preferred, the present investigation was undertaken with a view to developing a method based on the use of this oxidant, and capable of giving constant results over a wide temperature range. Attempts were also made to reconcile the disagreement among previous workers concerning the temperature stability of the molybdo-vanado–phosphoric acid complex.

EXPERIMENTAL

Measurements throughout this investigation were made with a Spekker Absorptiometer Type H 760 (Hilger & Watts Ltd.) under the following conditions:

Glass cells, optical path 4 cm.
Tungsten-filament lamp.
Kodak violet filters No. 543 (peak transmittance at 430 mμ).
Water setting on drum, 1.000.

In this instrument, the absorbance of the colored solution is referred to that of water, and the lower the drum reading, the greater the absorbance of the solution.

Examination of Color Complex in Absence of Iron. Development of the color complex in a solution containing only orthophosphate and nitric acid (with no oxidation) was first carried out in conjunction with a blank containing neither vanadate nor molybdate. For this and subsequent experiments a concentration of 0.4 mg. of phosphorus (as orthophosphate) per 100 ml. of final solution was employed. Coloring reagents were those specified by Kitson and Mellon: ammonium vanadate solution, 0.25%, 10 ml.; ammonium molybdate solution, 5%, 20 ml.

The acid concentration after dilution to final volume was 1.2*N*.

Under the above conditions (see Figure 1) the color complex becomes slightly more intense with increasing temperature. This is in accord with the work of Barton (*1*) but not with that of Hill (*6*), who claimed that the intensity of the color complex was independent of temperature.

The next point investigated was the effect of acid concentration on color complex intensity at various temperatures. For

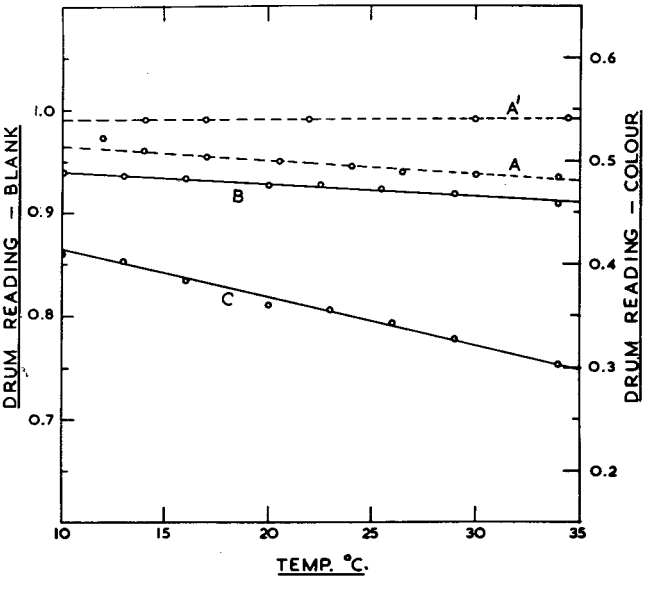

Figure 1. Effect of temperature on blank and color complex in absence of sulfate

A'. Background or blank in absence of ferric salts
A. Color complex in absence of ferric salts
B. Iron background
C. Color complex in presence of ferric salts

In Figures 1, 3, and 4 drum readings for color and blank have been brought into close proximity by the use of overlapping scales. All blanks refer to left-hand scale; all color readings refer to right-hand scale.

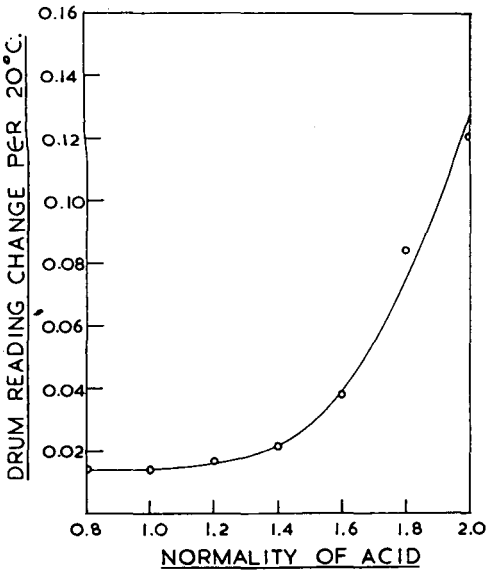

Figure 2. Temperature variation of color complex with acid concentration

this purpose, the tests were repeated for acid concentrations ranging from 0.2 to 2.0*N*. Beyond 2.0*N*, color development was inconveniently slow. The results showed that the intensity of color decreased with increasing acid concentration; the effect, however, was small below 1.6*N*, a result which is in substantial agreement with the work of Kitson and Mellon (*7*).

From these results a curve was plotted relating change of drum reading per 20° C. with acid concentration (see Figure 2); results below 0.8*N* were erratic and have accordingly been omitted. The curve shows that the change in color intensity of the complex with temperature is relatively small from 0.8 to 1.2*N* but rises sharply up to 2.0*N*, being marked at the latter concentration.

Consideration of the above results indicates that the optimum acid concentration in the final solution should be approximately normal, with respect to both maximum color development and minimum variation with temperature.

Examination of Color Complex in Presence of Iron. The color complex developed as before, but in the presence of 0.5 gram of high purity iron (Hilger spectrographic rods) shows both an intensification and a marked variation with temperature (Figure 1, *C*). This effect persists even after the subtraction of the ferric nitrate background color or blank, *B*, yet in the analysis of a steel by the method of Kitson and Mellon (modified by the incorporation of a blank) the results of Hill were confirmed—viz., that after deduction of the blank, the difference reading, or absorbance of the color complex, became independent of temperature.

The essential difference between the development of the complex in a pure iron solution to which additions of orthophosphate have been made, and the development of the color in an actual analysis of steel, lies in the necessity for an oxidation to convert the phosphorus in the steel to orthophosphate.

Effect of Oxidation Technique. A number of published variations of the method based on the formation of molybdo-vanado-phosphoric acid complex involving different oxidation techniques were next examined with respect to the dependence of the results on temperature.

The following techniques, each the subject of a previous paper, have been considered:

A. Method of Hill. (1) permanganate oxidation, (2) persulfate oxidation.
B. Method of Harrison. Oxidation by evaporation with brominated hydrochloric acid.
C. Method of Center and Willard. Oxidation by fuming with perchloric acid. This method as originally published was applied only to the determination of phosphorus in iron ores.

Oxidation with hydrogen peroxide was also examined, but had to be abandoned on account of the presence of phosphoric acid as a stabilizer in this reagent.

Throughout this work, the appropriate amounts of pure iron together with 0.4 mg. of phosphorus (as disodium hydrogen phosphate solution) were used; the color complex was developed in each method in accordance with the procedures outlined.

The drum readings corresponding to the intensity of both blank and color solutions have been plotted against temperature for each method in Figure 3. In only one of the oxidation techniques—the persulfate method of Hill—is the difference in absorbance between color and blank independent of temperature.

Effect of Sulfate Ion. The possible effects of end products of the oxidation reaction on the variation of color complex with temperature were next investigated. The method of Hill utilizes 10 ml. of a 15% solution of ammonium persulfate, most of which is subsequently decomposed by boiling, leaving approximately 0.9 gram of ammonium sulfate and 0.6 gram

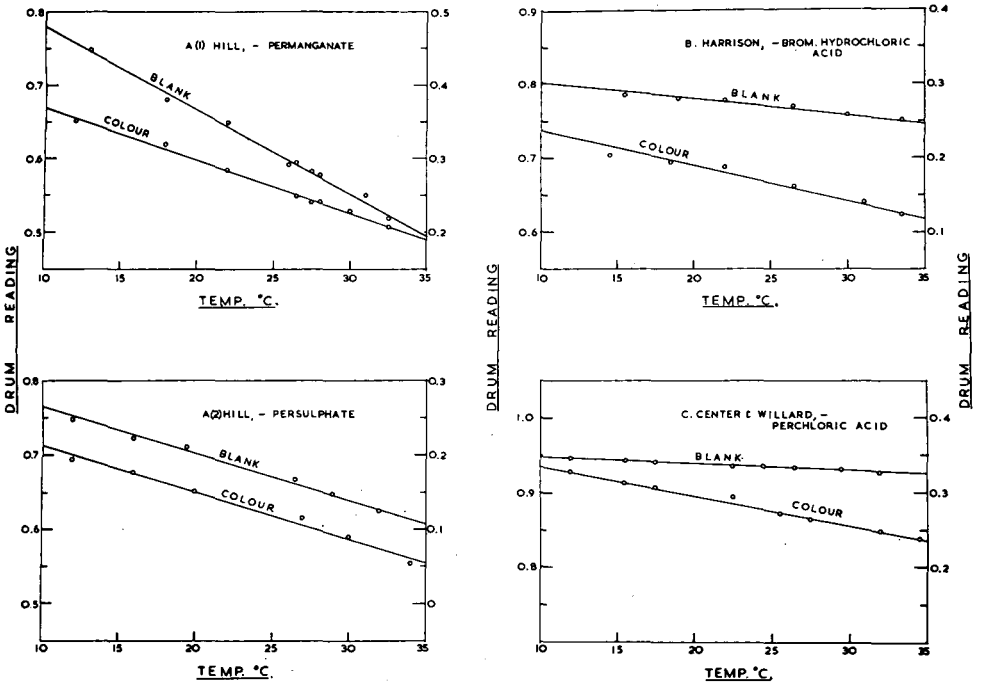

Figure 3. Examination of various methods involving different oxidation techniques

of sulfuric acid in the solution. (This additional acid is negligible in its effect on the final acid concentration.)

Accordingly the effect of 1-gram additions of pure ammonium sulfate was examined under the following conditions:

A. Color developed in the absence of iron.
B. Color developed in presence of 0.5 gram of iron.
C. Blank (0.5 gram of iron).

Nitric acid sufficient to give a final normality of 1.2, high purity iron, and 0.4 mg. of phosphorus (as disodium hydrogen phosphate solution) were again used throughout, but no oxidation other than boiling in nitric acid medium was carried out.

The results obtained showed that ammonium sulfate had no effect on the color complex in the absence of iron, but that both the iron background and the color complex plus iron background were intensified, in part by the formation of ferric sulfate which is more highly colored than ferric nitrate. Further, when transmittance was plotted against temperature (Figure 4) parallel lines were now obtained for color and blank, indicating that the addition of ammonium sulfate had enabled a constant transmittance difference to be obtained over the range of temperature investigated.

In the absence of sulfate the use of a blank will not ensure results independent of temperature (Figures 1 and 3), and the success of Hill's persulfate method can be attributed to the incidental introduction of ammonium sulfate as a by-product of the oxidation.

Additions of 0.5 gram of ammonium sulfate were insufficient to bring about the desired result, while larger amounts up to 2 grams were effective, but undesirable because of the increasing color of the iron background, which reduces the range of phosphorus that could be handled in the 4-cm. cells used in the Spekker Absorptiometer.

Similar effects were obtained with sodium sulfate, but not with ammonium nitrate, indicating that the "stabilizing effect" is brought about by the introduction of the sulfate ion.

APPLICATION OF RESULTS TO ANALYSIS OF STEELS

Having established that the fortuitous presence of sulfate ion was the factor responsible for the temperature-insensitiveness

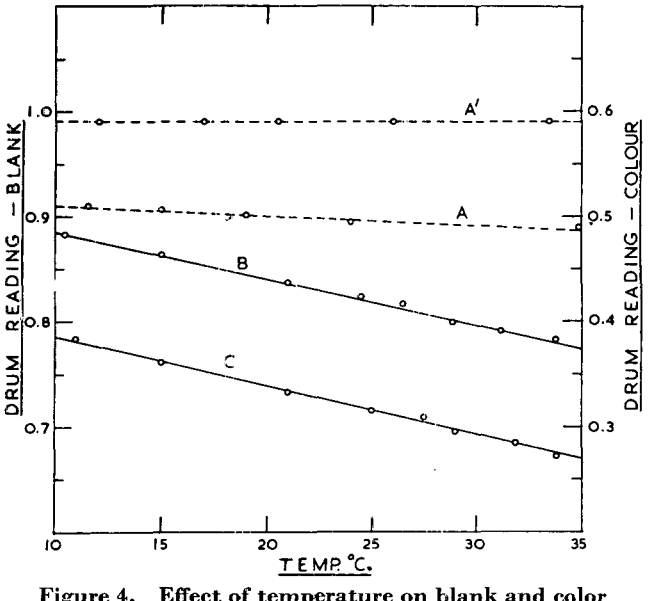

Figure 4. Effect of temperature on blank and color complex in presence of sulfate

A'. Background or blank in absence of ferric salts
A. Color complex in absence of ferric salts
B. Iron background
C. Color complex in presence of ferric salts

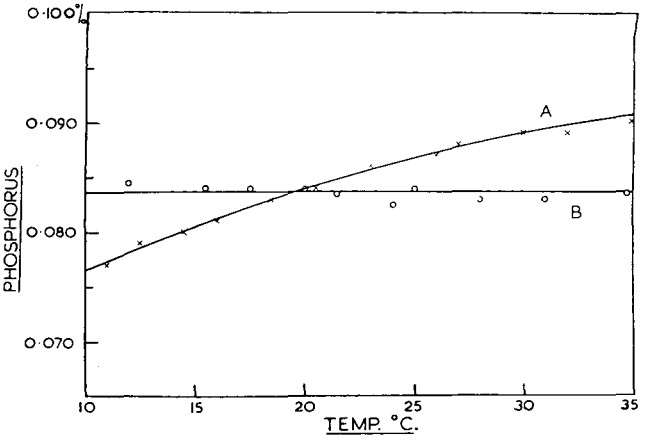

Figure 5. Variation of phosphorus result with temperature

NBS steel No. 22c, certificate value 0.083%
Authors' permanganate oxidation method
A. Without sulfate
B. With sulfate

of the persulfate method of Hill, it was considered likely that the temperature dependence characteristic of other methods of oxidation could be improved or even eliminated by deliberate addition of sulfate ion. The permanganate method, being the most convenient of available methods, was selected for trial.

In these tests, the "stabilizing effect" of sulfate ion was confirmed, and a method was developed in which the transmittance difference between color and blank was independent of temperature within the range 10° to 35° C.

Modified Permanganate Method. In the method developed, ammonium sulfate is added with the nitric acid used for dissolving the sample.

After solution and oxidation of the sample, two aliquots are used, one for color development and the other for the blank, thereby eliminating difficulties encountered by Murray and Ashley in obtaining reproducible iron background colors. A single coloring reagent containing both vanadate and molybdate is used, and, as neither vanadate nor molybdate is added to the blank, the method is applicable to alloy steels containing either vanadium or molybdenum. Sodium vanadate and sodium molybdate are preferred to the corresponding ammonium salts on account of their greater solubility.

Reagents Required. Nitric acid mixture, specific gravity, 1.160 at 20° C., to which have been added 40 grams of ammonium sulfate analytical reagent grade per liter of acid.

Potassium permanganate, 2% solution.

Sodium nitrite, analytical reagent grade.

"Color reagent," 3 grams of sodium vanadate and 100 grams of sodium molybdate made up to 1 liter with water, and filtered if necessary.

Disodium hydrogen phosphate, 0.4584 gram dried at 105° C. and dissolved in 1 liter of water.

Procedure. To 1 gram of the steel add 50 ml. of acid mixture, warm to dissolve, and boil until nitrous fumes have been expelled. Oxidize with 5 ml. of potassium permanganate solution and boil for 2 minutes.

Remove from the hot plate and clear the solution with the minimum quantity of solid sodium nitrite (approximately 0.05 gram is required). Again boil to expel nitrous fumes. Cool and dilute to the mark in a 100-ml. graduated flask. Pipet 50 ml. of this solution into a second 100-ml. graduated flask. To the original flask add 10 ml. of color reagent by means of a pipet, mix, and allow to stand for 10 minutes. Make up both color solution and blank to 100 ml. Measure the intensity of blank and color solutions at the same temperature, and from the difference reading obtained determine the percentage of phosphorus from the calibration graph.

Preparation of Calibration Graph. The calibration graph, to cover the range 0 to 0.10% phosphorus, was prepared by adding the appropriate amounts of disodium hydrogen phosphate solution

to samples of Hilger spectrographically pure iron of known phosphorus content, and proceeding exactly as set out in the method.

The resultant curve indicates a slight departure from Beer's law beyond a concentration of 0.06% phosphorus.

Notes. The method is independent of temperature, but it is essential that both blank and color solutions be brought to the same temperature prior to measurement. In the preparation of the calibration curve the difference in temperature between color and blank should not exceed 0.5° C., but slightly greater latitude may be allowed in the actual analysis of a steel.

Table I. Results of Analyses

No.	Type	Phosphorus Content, % Certificate value	Found	Temp., ° C.	Deviation, %
		NBS Standards			
11d	BOH	0.006	0.006	24	0.000
13e	BOH	0.021	0.021	16	0.000
21d	AOH	0.041	0.043	14	+0.002
20c	AOH	0.055	0.055	25	0.000
22c	Bessemer	0.083	0.083	28	0.000
129a	Bessemer	0.094	0.094	24	0.000
32d	Nickel-chromium	0.012	0.013	18	+0.001
106a	Nitralloy G	0.016	0.015	27	−0.001
33c	Nickel	0.017	0.016	20	−0.001
130	Lead-bearing	0.025	0.026	30	+0.001
156	Nickel-chrome-molybdenum	0.032	0.031	22	−0.001
		NPL Standard			
3		0.029	0.028	2	−0.001
		BCS Standards			
150	BOH iron	0.008	0.008	25	0.000
215	0.9% carbon	0.038	0.040	12	+0.002

Acid concentration is not highly critical, but the volume of acid mixture used to dissolve the sample should be controlled within ±2 ml. of the specified volume.

Some steels contain carbides resistant to attack by the acid mixture used. In such cases, dissolve the sample in 10 ml. of (2 to 1) nitric acid, evaporate almost to dryness, add 50 ml. of the nitric acid ammonium sulfate mixture, and proceed as outlined in the method.

The temperature insensitivity of the modified permanganate oxidation method has been demonstrated using National Bureau of Standards steel No. 22c. The results over the range 10° to 35° C. are shown in Figure 5 and are compared with the results obtained in the absence of sulfate. Separate calibrations were necessary, the results in the absence of sulfate being read from a calibration curve prepared at 18.5° C.

Both the accuracy of the method and its applicability to a wide range of steels have been checked by analyzing a series of standards; the results, together with the temperatures at which the optical measurements were made are set out in Table I.

These results, obtained at widely different temperatures from a single calibration curve, provide confirmatory evidence of the insensitivity of the method to temperature. Furthermore, the close correlation of the results with the certificate values suggests that the method is at least as accurate as the best of other available methods.

ACKNOWLEDGMENT

The paper is published with the permission of the Chief Scientist, Department of Supply, Australia.

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Routine Energy Measurements of Soft Radiations

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Identification of one or more radioisotopes in a mixture frequently may be established by determination of the energy of emitted particles. Energy determinations are made by means of absorption studies, utilizing absorbers positioned inside of a proportional chamber having a geometry of 2π. Very low energy levels can be measured, limited only by the thinnest absorbers which can be fabricated. Beta particles can be distinguished from α radiation by changing the voltage setting of the instrument. With this system the energy of numerous very soft β particles, as well as hard β particles, has been measured in the presence of α radiation—for example, the energy of the 29 k.e.v. radium-D β in the presence of hard β and α particles; the lead recoil atom from α emission of polonium-210 has been identified by means of energy determinations; and *K*-capture x-rays in a mixture have been identified.

FREQUENTLY, identification of individual isotopes of an element is required, particularly where the element is separated as the result of a chemical analysis. Usually, identification of a radioactive isotope can be made if the maximum range of the emitted particles can be determined, and the maximum energy may be calculated from the range of the particle.

Feather (*7*), Glendenin (*8*), and others have developed techniques for determination of β energy by means of absorption studies using Geiger counters. Their methods require comparatively large amounts of activity and β energies exceeding 0.1 m.e.v. for reliable results. A method capable of measuring the energy of very soft radiation in the presence of hard emission in samples of low total activity is of value (*4*, *5*). Such a method has been developed, using a windowless absorption counter (*1–3*, *11*) and absorbers (*3*, *12*).

COUNTER

The counter consists of a slide mechanism (Nuclear Measurements Corp.) with a Type CC-2, 2-inch hemispherical proportional chamber and a Model P-2 piston, a conventional scaler, and a high-gain, wide dynamic-range amplifier (*1*, *2*). The piston was modified so that absorbers could be inserted into the sensitive volume of the chamber between the sample and the center wire. This modification consisted of a depression formed in the piston to hold the sample and a ledge around the depression to support the absorbers. During counting, P-10 gas, consisting of 90% argon and 10% methane, was circulated through the chamber over the sample; α particles were counted at a definite voltage

and α plus β particles and γ rays were counted at a higher voltage.

A series of supporting rings and absorbers (*12*) was so made that the total thickness of ring and absorber was 0.25 inch. The absorbers ranged in thickness from 0.02 to 1700 mg. per sq. cm. The thinnest absorbers, 0.02 to 0.10 mg. per sq. cm., were made from Formvar E film and rendered electroconducting by evaporating silver or aluminum onto the film. Electroscope foil, in single layers and in two, three, or four laminations, and commercially available aluminum foil were used for the range from 0.15 to 35.0 mg. per sq. cm. The thin absorbers were cemented to the supporting rings. The thicker absorbers were machined from round aluminum stock 2 inches in diameter, the ring and absorber being formed as a complete assembly.

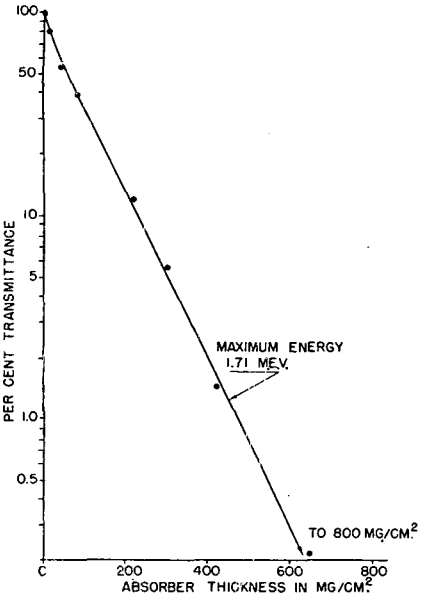

Figure 1. Absorption curve of phosphorus-32

The distance from the bottom of the absorber to the sample must be maintained at a constant value, and must be as small as possible in order to minimize geometry loss and gas absorption effects. A set of spacers was made, one spacer for each absorber, of a thickness such that a distance of 0.05 inch was maintained between the top of the spacer and the bottom of the absorber when these parts were assembled in the depression formed in the piston. Sample mounts were placed on top of the spacer, and when thin mounts were used, a shim was put under the spacer to bring the sample as close as possible to the absorber.

PROCEDURE

Measurements are made by placing the sample in position, flushing the chamber with gas, and counting the samples with gas flowing through the chamber. This procedure is repeated with selected absorbers in place over the sample. When β radiation is counted in the presence of α emission, an α measurement is made followed by an α-plus-β measurement. The difference in the two measurements is recorded as the β count. The efficiency for γ radiation is very poor, being about 1% for a cobalt-60 γ, 1.2 m.e.v.

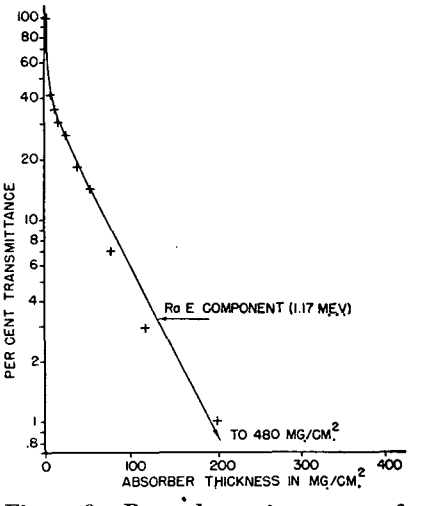

Figure 3. Beta absorption curve of radium D-E-F

The percentage transmittance of each β-plus-γ measurement with background subtracted is plotted against absorber thickness on semilog paper. The equivalent of the absorption of the gas between the sample and the absorber must be added to the aluminum-absorber thickness. The maximum range is determined from this graph, and the maximum energy is determined from the range, using the Glendenin range-energy relationships (*10*).

In determining the maximum range from the graphs, energy studies on standards with a maximum energy approaching that of the unknown are of material assistance. Carbon-14 (0.16 m.e.v.), radium E (1.17 m.e.v.), and phosphorus-32 (1.71 m.e.v.) are suitable for use as standards. The absorption curve of a single β emitter, as determined by the windowless absorption counter, approximates a straight line over most of the range (Figures 1 and 2). A Feather analyzer (*9*), prepared from the absorption curve of an appropriate standard, is placed along the vertical scale of the absorption curve of the unknown, with the zero reading on the analyzer coinciding with 100% transmittance on the absorption curve. The point on the vertical scale which corresponds to 1.0 on the analyzer is taken as substantially zero transmittance. The absorber thickness required to reach this point, as determined from the extrapolated absorption curve of the unknown, is the maximum range of the unknown. This technique has been tested with a number of emitters of known energy, and it has been found to be a sound procedure. If several components are present in a sample, the contribution of the hardest component may be subtracted from the curve and the energy of the second component determined. The contribution of this component may be subtracted from the remainder to obtain softer components, until all components have been determined.

Since there is no window for the radiation to penetrate, the energy levels which can be measured are very low, being limited

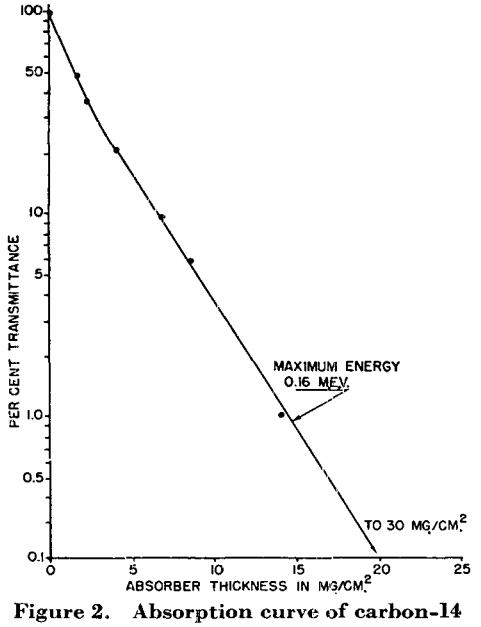

Figure 2. Absorption curve of carbon-14

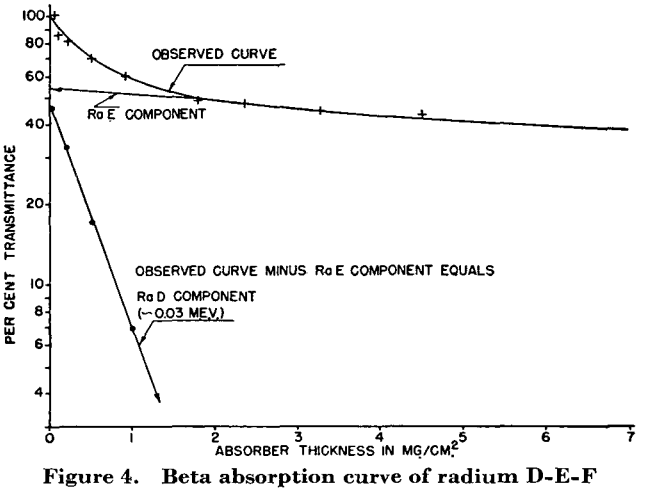

Figure 4. Beta absorption curve of radium D-E-F

only by the thinnest absorber which can be fabricated. The geometry is favorable (2π), so that a very small amount of activity is required.

RESULTS AND DISCUSSION

Figures 3 and 4 show the absorption of β particles from radium-D (~0.03 m.e.v.) and radium-E (1.17 m.e.v.) counted in the presence of the radium-F α (5.3 m.e.v.). Figure 5 is the absorption curve of nickel-63 (0.07 m.e.v.); Figure 6 is the curve of a mixture of sulfur-35 (0.17 m.e.v.) and nickel-63; Figure 1 is the absorption curve of phosphorus-32 (1.71 m.e.v.); and Figure 2 is the curve of carbon-14 (0.16 m.e.v.).

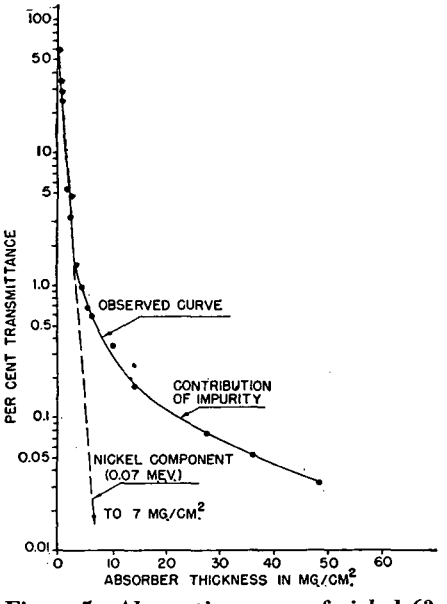

Figure 5. Absorption curve of nickel-63

The absorption of K-capture x-rays from iron-55 is shown in Figure 7. The steep initial drop of the sample mounted on tantalum is not present in the same material mounted on glass. This is attributed to absorption of very weak secondary electrons formed in the high-atomic-number backing material by the x-rays. These x-rays can be distinguished from soft β particles, since a soft β absorption curve does not appreciably change with backing material. This method is much simpler than the customary one of changing the atomic number of the absorber as only one set of absorbers is required.

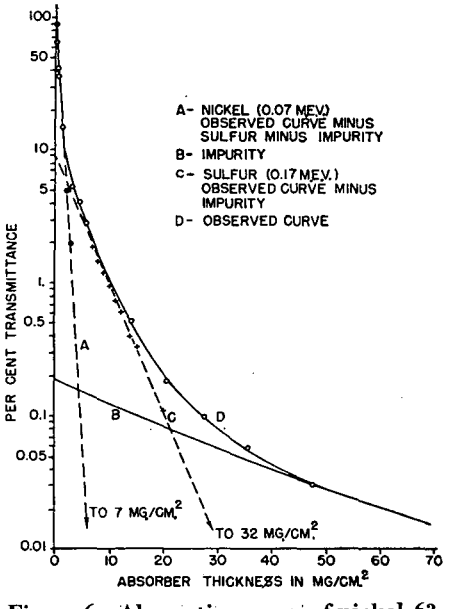

Figure 6. Absorption curve of nickel-63 and sulfur-35

The windowless absorption counter was used to identify recoil atoms from α emission. These were being counted as β particles in samples of polonium-210 and plutonium-239 which were known to contain no appreciable amounts of β activity. In sufficiently thin samples, one apparent β was counted for each α, and the half life was found to be the same as that of the α.

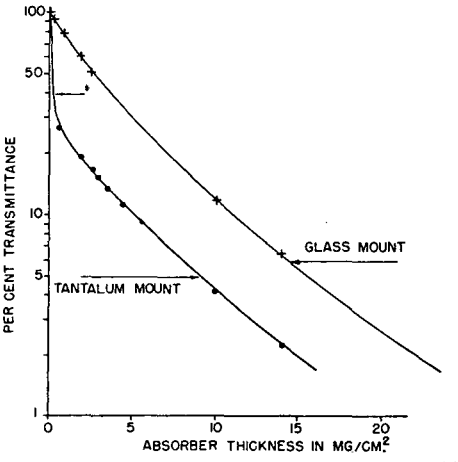

Figure 7. Absorption curve of iron-55

*** Absorption of secondary electrons formed in tantalum by K-x-rays from Fe^{55}**

Absorption of the apparent β associated with the α decay of polonium is shown in Figure 8. It was assumed that the range of the recoil atom, kinetic energy 0.96 m.e.v., would be less than that of an α particle of the same energy, or 0.15 mg. per sq. cm. of aluminum. The maximum range of approximately 0.08 mg. per sq. cm. in Formvar, calculated from Figure 8, corresponds to the expected value and confirms the identification of lead recoil atoms. The tail on the absorption curve of Figure 8 has not been satisfactorily explained, but it has been observed repeatedly. It may be due to L x-rays from the recoil of excited lead atoms, but the authors are unable to give a positive explanation.

In measuring soft radiation, samples should be as nearly weightless as possible. The samples used in this study were prepared by pipetting carrier-free solutions onto slides. Metal disks were used because best results are obtained with electro-conducting mounts. The metal used for a given sample must not be attacked by solvent in which the isotope is dissolved. If the solution to be analyzed is not entirely carrier-free self-absorption is not a serious problem; because of the favorable counter geometry, a dilute solution can be used.

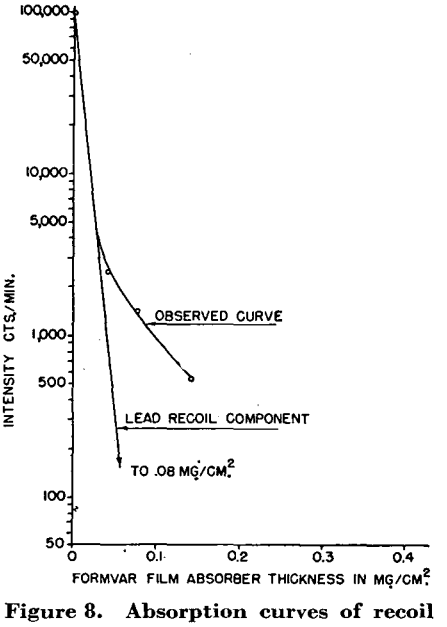

Figure 8. Absorption curves of recoil atoms

This counting system has been used extensively in identification of unknown emitters. For example, chemical tests indicated the presence of radioactive iron in a solution. An absorption

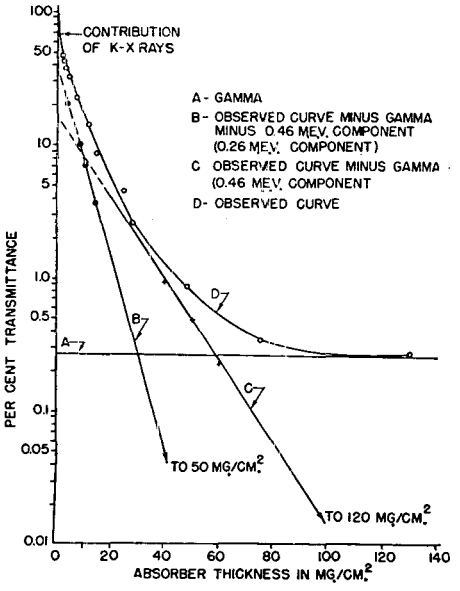

Figure 9. Absorption curve of mixture of iron-55 and iron-59

study of the iron separation was made. Analysis of the curve shown in Figure 9 clearly shows the γ and the 0.26- and 0.46-m.e.v. β particles from iron-59 and the K-capture x-rays from iron-55. A half-life study confirmed the identification.

The application of this system to α counting has been described (*6*). It is shown that the absorption curve of monoenergetic α particles when plotted on linear paper is a straight line whose intercepts are the range of the particles and the absolute α count. This makes possible very precise energy measurements of α emitters. The method is also applied to determining the proportion of two α emitters in a mixture.

These techniques are not infallible in identifying unknown emitters in a mixture, but they can be extremely useful when used in conjunction with chemical analysis and half-life studies.

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Schiff Reagent

Its Preparation and Its Use in the Determination of Formaldehyde in Cellulose Acetate Formal

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This work was undertaken to provide a method for the determination of small amounts of combined formaldehyde in cellulose acetate formal samples. A Schiff reagent of controlled sensitivity and improved stability has been developed and utilized. Sensitivity has been correlated to sulfur dioxide concentrations and both optimum and reproducible response are shown to occur in the range 2.8 to 4.8 millimoles of sulfur dioxide per 100 ml. of reagent. The formaldehyde content of cellulose acetate formals has been determined colorimetrically using Schiff reagent. The method developed shows a standard deviation of 0.021% of formaldehyde for samples of cellulose acetate formal film containing less than 0.8% of formaldehyde. The improved performance of Schiff reagent has resulted in an analytical tool of increased versatility. Sufficient stability and reproducibility have been obtained to make daily calibration unnecessary.

THE development of a colorimetric method for the determination of formaldehyde in cellulose acetate formal falls naturally into two parts: the preparation of a sensitive Schiff reagent which will give reproducible results against known amounts of formaldehyde, and the achievement of sample hydrolysis in such a way that the formaldehyde is quantitatively obtained in a form suitable for measurement with Schiff reagent.

STUDY OF SCHIFF REAGENT

Blaedel and Blacet (*1*) found that Schiff reagent reacts with formaldehyde in strong sulfuric acid to give a blue color which reaches a maximum intensity after 2 to 2.5 hours. While other aldehydes may also produce initial colors, these colors fade completely in the 2- to 2.5-hour period prescribed. This makes the test selective for formaldehyde. Blaedel and Blacet applied it to the determination of formaldehyde in the presence of other aldehydes. Hoffpauir, Buckaloo, and Guthrie (*2*) adapted the

procedure of Blaedel and Blacet for use with a photoelectric colorimeter, and obtained a reproducible curve for transmittance (at 550 to 585 mμ) against milligrams of formaldehyde. This curve, however, is S shaped and does not obey the Beer-Lambert law.

Several initial attempts to reproduce the work of Hoffpauir and others were unsuccessful. This was primarily due to the difficulties encountered in preparing a colorless Schiff reagent of the required sensitivity. Other workers, notably Segal (*4*), have encountered similar difficulties.

In view of these difficulties, a quantitative study was made of the following factors affecting Schiff reagent preparation and sensitivity: influence of rosaniline hydrochloride concentration, influence of sulfur dioxide concentration, and conditions for decolorizing with activated carbon.

Apparatus: Lumetron colorimeter Model 402-E, Photovolt Corp. (or equivalent) 550-mμ glass filter.
Absorption cells 0.5, 1, and 2 cm. (Photovolt Corp.).

Reagents. Rosaniline hydrochloride, Matheson, Coleman, and Bell.
Sodium meta bisulfite, reagent grade ($Na_2S_2O_5$).
Activated carbon, Nuchar-CN (West Virginia Pulp & Paper Co.).
Sulfuric acid, 6*N*.
Standard iodine solution, 0.1*N*.
Starch solution, 0.5%.
Formalin (40% aqueous formaldehyde solution).
Mixed acid reagent, volume composition 50% water, 5% 10*N* hydrochloric acid, 45% 14*N* sulfuric acid.
Aqueous hydrochloric acid, 10*N*.
Aqueous sulfuric acid, 14*N*.
Schiff reagent prepared as designated below.

Preliminary work with various batches of Schiff reagent, prepared according to the specifications of Hoffpauir *et al.* (*2*) and decolorized with activated carbon as described by Segal (*4*) indicated that an optimum concentration of 3.0 millimoles of sulfur dioxide per 100 ml. of reagent gave greatest sensitivity. These reagents, as those of most previous investigators, contained 100 mg. of basic fuchsin per 100 ml. In order to see whether any worth-while gain in sensitivity could be attained by increasing the dye concentration, several high dye reagents were prepared, with the sulfur dioxide concentration controlled in accordance with the previous finding. Sensitivity was assessed by reacting each reagent with known amounts of formaldehyde and measuring the transmittance after color development. Results are summarized in Table I.

Table I. Influence of Dye Concentration on Schiff Reagent Performance

Dye, Mg./100 Ml.	SO_2, Millimoles/ 100 Ml.	HCHO, Mg. 0.023	0.046	0.077	0.131
		Per Cent Transmittance at 550 Mμ, 2-Cm. Cell			
100	3.01	97.6	94.8	83.0	...
200	3.04	97.3	94.6	79.1	43.7
300	2.84	95.3	93.3	74.8	35.5

It is apparent from Table I that worth-while gains in reagent response are achieved in going from dye concentrations of 100 mg. per 100 ml. to concentrations of 300 mg. per 100 ml. The colors obtained using reagents with dye concentrations greater than 300 mg. per 100 ml. appear to be dichroic, having purple to red hues instead of the usual blue color obtained with reagents of lower dye concentrations. Work with these reagents was therefore limited to this qualitative observation.

In view of the above results it was decided to base further work on a Schiff reagent containing 300 mg. of dye per 100 ml. Since the optimum sulfur dioxide content previously established as 3.0 millimoles of sulfur dioxide per 100 ml. of Schiff reagent applies to a reagent containing only 100 mg. of dye per 100 ml., this experiment was repeated to establish the sulfur dioxide concentration limits for optimum response of the high dye reagent. These results are plotted in Figure 1.

Reference to Figure 1 indicates that in the range 2.8 to 4.8 millimoles of sulfur dioxide per 100 ml., reagent response is essentially a maximum, and that above and below this range, reagent response rapidly deteriorates. It is, therefore, proposed to hold the sulfur dioxide content within this optimum range.

Trial and error experimentation in the decolorization of Schiff reagent with activated carbon has shown that both the amount of carbon used and the reagent-carbon contact time exert important influences on the sensitivity of the reagent. In general, the use of small amounts of carbon (200 to 800 mg. per 500 ml.) and relatively long contact periods (5 to 15 minutes) are not so effective as the use of larger amounts of carbon acting for shorter

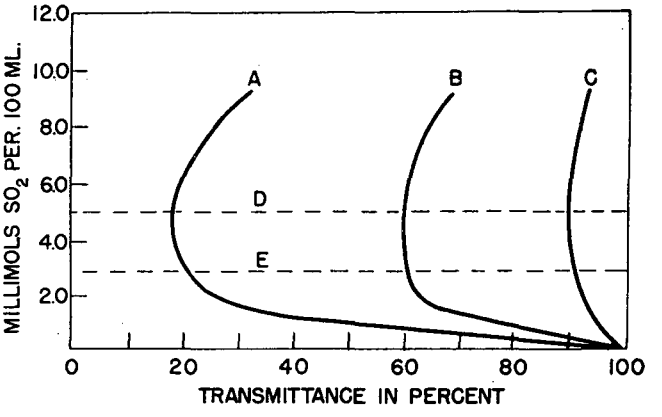

Figure 1. Loci showing variation of Schiff reagent sensitivity with reagent sulfur dioxide content for fixed formaldehyde levels

A. **0.250 mg. of HCHO**
B. **0.125 mg. of HCHO**
C. **0.050 mg. of HCHO**

Ordinates *D* and *E* enclose zone of optium reagent response

periods. Segal (*4*) recommends the use of 1 gram of carbon per 500 ml. of Schiff reagent and both Segal (*4*) and Tobie (*5*) use gravity filtration through filter paper to separate activated carbon, without specifying contact time. By employing a Büchner funnel and vacuum filtration, it has been found that much shorter reagent-carbon contact periods may be achieved. Presumably previous workers have avoided vacuum filtration in order not to lose sulfur dioxide. However, this fear is groundless once the optimum range for sulfur dioxide is known, for it is always possible to adjust the concentration after vacuum filtration, although experience has shown this to be rarely necessary. In practice, therefore, a 45-second contact period has been empirically developed and used at Segal's recommended carbon concentration of 1 gram per 500 ml., followed by a vacuum filtration which requires about 2, and no more than 3, minutes for completion.

Preparation of Schiff Reagent. Place 1500 ml. of distilled water in a 3-liter Erlenmeyer flask, add 4.500 ± 0.005 grams of rosaniline hydrochloride, and swirl to solution. Add 9.60 ± 0.05 grams of sodium metabisulfite, mix and let stand 5 to 10 minutes, then add 40 ml. of 6*N* sulfuric acid, mix well, stopper, and allow to stand overnight. Prepare a large Büchner funnel and 2-liter filter flask for a suction filtration through two sheets of No. 2 filter paper. Add 3.0 grams of activated carbon to the Schiff reagent, quickly mix by swirling, and time for a contact period of 45 seconds. After the lapse of the 45-second contact period, pour the Schiff reagent into the Büchner funnel, and filter with suction as rapidly as possible. The total time required for filtration should be about 2 minutes and no longer than 3 minutes. The above time specifications provide that all of the Schiff reagent will be in contact with the activated carbon at least 45 seconds and that none of the Schiff reagent will be in contact with the activated carbon longer than 225 seconds.

Pipet 10.0 ml. of the decolorized Schiff reagent into a 125-ml. Erlenmeyer flask, add 20 ml. of distilled water and 5 ml. of starch, and titrate the free sulfur dioxide to a starch end point with standard 0.1N iodine solution. Calculate the free sulfur dioxide as follows: 5 × milliliters of iodine × normality = millimoles of sulfur dioxide per 100 ml. of Schiff reagent. If the reagent sulfur dioxide content falls outside the optimum range of 2.8 to 4.8 millimoles of sulfur dioxide per 100 ml. of reagent, adjust it to higher or lower levels until a value within this range is attained. Sulfur dioxide concentrations may be raised by adding a calculated amount of sodium metabisulfite, and lowered by bubbling air through the reagent. In general, if the above steps have been followed, the reagent sulfur dioxide content will fall within the optimum range.

Standardization of Schiff Reagent. Prepare a dilute solution of formaldehyde containing about 8 mg. of formaldehyde per 100 ml. by adding 0.4 ml. of formalin to 2 liters of 0.05N sulfuric acid. This solution may be standardized by the gravimetric dimedon method of Yoe and Reid (*6*) or the modified bisulfite method employed by Nitschmann and Hadorn (*3*); in the present work the bisulfite method was employed. From the standard formaldehyde solution prepare by accurate dilution with 0.05N sulfuric acid at least ten standard formaldehyde solutions containing from 0.15 to 4.0 mg. of formaldehyde per 100 ml. To complete the standardization react 10 ml. of each formaldehyde solution with 20 ml. of Schiff reagent and 20 ml. of mixed acid reagent. After 2.0 to 2.5 hours determine the transmittance, in per cent, at 550 mμ, with the colorimeter. The useful range of the curve can be extended by using a 2-cm. cell for the low formaldehyde range, a 1-cm. cell for the intermediate range, and a 0.5-cm. cell for the high range, as indicated by Figure 2. For these measurements the instrument is adjusted for 100% transmittance with a blank containing 20 ml. of Schiff reagent, 20 ml. of mixed acid reagent, and 10 ml. of water.

Plot the transmittance in per cent *vs.* milligrams of formaldehyde on regular graph paper. Typical graph is shown in Figure 2. The batch to batch reproducibility of Schiff reagent prepared as indicated is illustrated by the transmittance data of Table II.

Table II. Standardization Data for Two Typical Batches of Schiff Reagent in Per Cent Transmittance at 550 Mμ

Mg. HCHO	0.5-Cm. Cell Batch A	0.5-Cm. Cell Batch B	1-Cm. Cell Batch A	1-Cm. Cell Batch B	2-Cm. Cell Batch A	2-Cm. Cell Batch B
0.417	11.4	11.3	..	..	..	..
0.334	21.5	22.2	5.4	5.7	..	..
0.250	39.0	39.5	16.6	17.0	..	..
0.209	50.2	52.0	26.0	27.0	..	..
0.167	..	..	40.6	41.2	..	..
0.142	..	..	51.1	51.7	..	..
0.125	..	..	58.3	59.4	..	..
0.109	..	..	66.3	66.8	..	..
0.084	..	..	77.0	77.6	..	..
0.067	..	..	83.9	85.0	..	..
0.050	..	..	90.0	90 7	80.8	81.6
0.034	..	..	..	..	90.1	90.7
0.025	..	..	..	..	94.1	94.7
0.017	..	..	..	..	96.7	97.5

Previous workers have employed a color development period of 2.0 to 2.5 hours for the Schiff reagent–formaldehyde reaction. The validity of this reaction time for the high dye reagent has been corroborated by determining transmittance as a function of time.

Contrary to the experience of Hoffpauir *et al.* (*2*), it has not been necessary to run a standard curve each time Schiff reagent is used. Standardization every 4 or 5 days is ample, provided the sulfur dioxide concentration is controlled within the optimum range. It is necessary, however, to run a standard curve each time a new lot of rosaniline hydrochloride is received or when the source of the dyestuff is changed. A change in source of dyestuff may produce minor changes in the shape and placement of the standard curve. The data of Table III, collected over a period of 23 days for a single batch of Schiff reagent, show the degree of stability that may be expected in reagent response.

When the reagent was between 13 and 17 days old, it was found that the sulfur dioxide concentration had fallen below the optimum range of 2.8 to 4.8 millimoles of sulfur dioxide per 100 ml. Therefore, it was adjusted upward by the addition of sodium metabisulfite; thereafter the reagent response remained essentially constant from ages 17 to 23 days. The maximum error shown by the preceding data, for standardizations no more than 6 days apart, is about 6% of the formaldehyde measured. It is felt that the degree of stability of reagent response demonstrated above is due primarily to control of sulfur dioxide concentrations within the optimum range. Freedom from the necessity of standardizing Schiff reagent daily provides a method of greater utility than that previously obtained. No special precautions were taken to preserve the Schiff reagent used to collect the above data. The reagent was stored in a clear glass carboy under air with a rubber stopper closure.

Table III. Per Cent Transmittance Obtained for Schiff Reagent Aged 1 to 23 Days (1-Cm. Cell)

Mg. HCHO	1 Day	6 Days	13 Days	17 Days	20 Days	23 Days
0.050	90.0	90.1	89.6	91.0	91.1	91.0
0.109	66.3	66.7	72.4	69.0	70.2	69.3
0.142	51.1	51.2	54.1	54.6	56.0	55.1
0.209	26.0	27.2	29.5	29.2	30.7	30.4

Table IV. Solubility of Cellulose Acetate Formal in Hydrochloric Acid

Normality of HCl	Approximate Time Required to Dissolve 1 Gram C. A. Formal in 10 Ml. of Acid, Min.	Color of HCl Solution after 17 Hours	Effect of Subsequent Dilution with Distilled Water
12	15	Dark brown	No precipitation
10	30	Light yellow	No precipitation
8	100	Colorless	Polymer precipitates
6	Insoluble	Insoluble	Polymer precipitates

ANALYSIS OF CELLULOSE ACETATE FORMAL

Hoffpauir *et al.* (*2*), in the determination of formaldehyde in cellulose formals, hydrolyzed the samples by an overnight treatment with 12N sulfuric acid at room temperature. This procedure does not work in the case of cellulose acetate formals. Conditions were therefore varied by letting the sulfuric acid normalities range from 12 to 18N and hydrolysis temperature, from room temperature up to 50° C., without success.

Strong hydrochloric acid, on the other hand, was found to be a rapid acting solutioning and hydrolyzing agent for cellulose acetate formal. The results of trials at various hydrochloric acid normalities are shown in Table IV.

On the basis of the qualitative solubility behavior exhibited in Table IV, 10N hydrochloric acid was selected for further work. It was thought that the Schiff reaction could be carried out in strong hydrochloric acid solution as well as in strong sulfuric acid solution; however, this is not the case. When 10N hydrochloric acid is employed to replace the 12N sulfuric acid used by Hoffpauir *et al.* (*2*) in standardizing Schiff reagent, yellow to orange colors are produced instead of blue. It appears, therefore, that hydrochloric acid provokes an interference reaction of some kind, and that the presence of sulfuric acid is essential.

The high efficiency of hydrochloric acid as a sample hydrolyzing agent for cellulose acetate formal made its abandonment undesirable, consequently a means was sought to eliminate hydrochloric acid interference effectively. To this end, mixed hydrochloric-sulfuric acid solute systems were investigated as color developing media for Schiff reagent. It was hoped that the ratio of hydrochloric to sulfuric could be controlled so that the interference reaction would be inhibited and the normal color reaction would proceed. These systems are listed in Table V.

From the above work, it appears that the hydrochloric acid normality, rather than hydrochloric-sulfuric ratio, is the factor controlling interference. Furthermore, in color developing media over 0.2N in hydrochloric acid, interfering reactions predominate.

Solute system 4 has been adopted as the basis for the analytical method for formaldehyde in cellulose acetate formal. Several

Table V. Various Solute Systems as Color Developing Media for Schiff Reagent

System No.	10N HCl, Ml.	14N H_2SO_4, Ml.	H_2O, Ml.	$MgSO_4$ Solution[a], Ml.	Schiff Reagent, Ml.	HCHO Std., Ml.	Ratio Cl/SO_4	Comments
1	10.00	3.57	6.43	0	20.00	10.00	4.12	Unsatisfactory orange-yellow colors develop
2	1.00	7.72	11.28	0	20.00	10.00	0.24	Satisfactory except at very low HCHO concentrations
3	1.00	7.72	1.28	10.00	20.00	10.00	0.16	Satisfactory except at very low HCHO concentrations
4	1.00	9.00	10.00	0	20.00	10.00	0.16	Satisfactory over entire range of HCHO concentrations
5	1.50	13.50	5.00	0	20.00	10.00	0.16	Unsatisfactory orange-yellow colors develop
6	2.00	18.00	0	0	20.00	10.00	0.16	Unsatisfactory orange-yellow colors develop

[a] 680 grams per liter $MgSO_4$.

samples were allowed to react with 10N hydrochloric acid at room temperatures for time periods ranging from 17 to 67 hours. A constant maximum formaldehyde yield is obtained after 17 hours; at shorter time periods incomplete sample breakdown is achieved. About 5% of the samples encountered are incompletely broken down after 17 hours at room temperature. For these samples, a subsequent 2-hour low temperature heat treatment at 35° to 40° C. effectively completes sample decomposition. To establish uniform sample-to-sample degradation conditions, this heat treatment was adopted for all samples. Use of temperatures higher than 35° to 40° C. result in the formation of prohibitive amounts of extraneous color (brown to black).

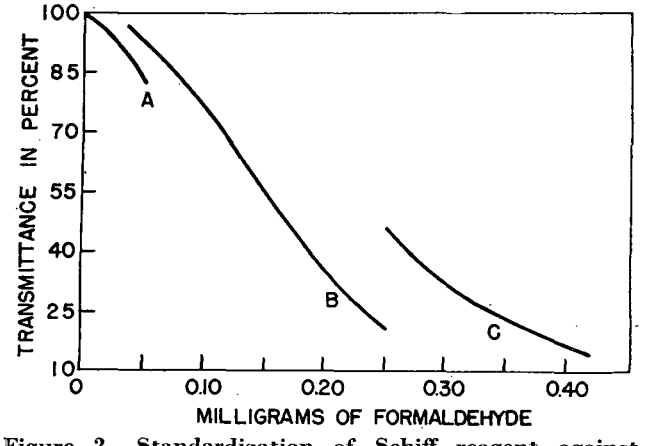

Figure 2. Standardization of Schiff reagent against known amounts of formaldehyde

A. 2.0-cm. cell
B. 1.0-cm. cell
C. 0.5-cm. cell

Analysis is accomplished by the following sequence of operations:

Overnight solution of a weighed sample in 10 ml. of 10N hydrochloric acid followed by a 2-hour heating period at 35° to 40° C.

Addition of 90 ml. of 14N sulfuric acid to provide a mixed acid hydrolyzate 9 to 1 in 14N sulfuric acid:10N hydrochloric acid.

Reaction of a 10-ml. aliquot of the above mixed acid hydrolyzate with 20 ml. of Schiff reagent and 20 ml. of water.

Determination of transmittance at 550 mμ and calculation of results.

Twenty-five different samples, ranging from 0.01 to 0.8% formaldehyde, were analyzed in duplicate. The data so obtained yield a standard deviation of 0.021% formaldehyde. This is considered good reproducibility, especially since duplicate determinations have been carried out on successive days rather than simultaneously. A further point of interest concerns the preparation of the standard curve for known amounts of formaldehyde. From an operational standpoint, it is desirable to determine this curve in the absence of hydrolyzed cellulose acetate. It has been shown experimentally that the standard curves with and without hydrolyzed cellulose acetate are identical and, therefore, the omission of acid hydrolyzed cellulose acetate from the standardization solute system is a valid shortcut.

PROCEDURE FOR ANALYSIS OF SAMPLES. Accurately weigh into a tared 125-ml. glass-stoppered Erlenmeyer flask a sample of cellulose acetate formal of appropriate size. If, as is generally the case, there is initially no knowledge of the formaldehyde level, choose an arbitrary sample size of 1.0 to 1.5 grams and carry out the procedure described below. In the event that the transmittance fails to fall within the range of 10 to 95%, consider the determination to be a pilot determination and adjust sample size to a satisfactory level in accordance with the indications of the pilot determination. In case the use of the arbitrary sample size provides a transmittance within the range 10 to 95%, accept the determination as a valid one.

To the weighed sample accurately add 10.00 ml. of 10N hydrochloric acid, stopper the flask, and swirl vigorously. During the first 2 hours, occasionally swirl the flask vigorously, then allow to stand overnight at room temperature (17 to 20 hours). Securely stopper the flask and heat for 2 hours at 35° to 40° C., cool to room temperature, then add from a 100-ml. buret, 90.00 ml. of 14N sulfuric acid and mix. Pipet a 10-ml. aliquot of the mixed acid hydrolyzate into a 125-ml. glass-stoppered Erlenmeyer flask containing 20.00 ml. of distilled water. In the event that this 10-ml. aliquot provides a prohibitively low transmittance (due to high formaldehyde content), a smaller aliquot may be employed instead. Optional aliquot sizes to meet this contingency, together with accompanying changes in color developing media composition, are indicated in Table VI. Add by automatic pipet 20.0 ml. of Schiff reagent, mix, and let stand for 2 hours. (Caution. The use of suction in pipetting Schiff reagent will result in undesirable loss of reagent sulfur dioxide.) Measure the transmittance in per cent of the colored solution at 550 mμ with a colorimeter. Adjust the instrument for 100% transmittance with a blank containing 20 ml. of Schiff reagent, 20 ml. of mixed acid reagent, and 10 ml. of distilled water. From the transmittance obtained, determine the milligrams of formaldehyde by reference to the standard curve. Calculate the formaldehyde content of the sample as follows:

$$\% \text{ HCHO} = \frac{\text{milligrams of HCHO from curve} \times 10}{\text{aliquot size in milliliters} \times \text{sample wt. in grams}}$$

The use of an aliquot greater than 10 ml. will result in an interference reaction involving hydrochloric acid.

Table VI. Composition of Color Developing Media for Various Size Aliquots

Aliquot Size, Ml.	Mixed Acid Reagent, Ml.	H_2O, Ml.	Schiff Reagent, Ml.	Total Volume Colored Solution, Ml.
10.00	None	20.00	20.00	50.00
5.00	10.00	15.00	20.00	50.00
3.00	14.00	13.00	20.00	50.00

Limited work performed subsequent to the development of the above procedures has indicated that the inclusion of acetic acid in the reaction medium enhances the sensitivity of the reagent, especially at low formaldehyde concentrations. Specifically, 10 ml. of water in the reaction system were replaced with 10 ml. of glacial acetic acid.

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Stable Cholinesterase Preparations as Laboratory Standards of Activity

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Stable enzymatic standards for cholinesterase activity have been developed on filter paper disks using bovine red cell cholinesterase as the enzyme source. When stored in the refrigerator over desiccant, the disks retain their activity for at least 6 months. The enzyme activity may be eluted from these disks by solutions employed in any of the common procedures for the determination of cholinesterase activity. Since the cholinesterase activity found for the disks by any of these procedures varies by only 2 to 3%, the disks may be used as a reproducible standard source of cholinesterase activity for comparison between different analytical methods, or as a control on the reliability of a single method.

THE use of various methods for the determination of cholinesterase activity has led to some confusion as to how activities measured by one procedure can be connected with or interpreted in terms of another. For example, cholinesterase activity has been measured in various laboratories, in terms of ΔpH per hour by the electrometric method (*8*), microliters of carbon dioxide in a given time by the manometric method (*2*), milliliters of standard alkali by the constant pH procedure (*6*), and micromoles of acetylcholine hydrolyzed in unit time by the colorimetric method (*5*). The activity measured is generally not the same from one method to the other because the various procedures differ with respect to time, temperature, substrate, salt concentration, and other factors affecting enzyme activity. Variable results within any one method may be due to real changes in the enzyme activity or to variability in the procedures applied for measuring that activity. A similar problem in usual analytical procedures is solved by the use of reproducible standards which can be made from the chemically pure substance and analyzed along with an experimental sample. Cholinesterase is not available as a chemically pure substance, and the enzyme has to be measured in terms of its activity. But if units of reproducible constant activity could be made, these could be used as standards to ascertain the source of variation in a given method for determining cholinesterase activity where variable results were being obtained. Moreover, the different methods of measuring enzyme activity could be compared with one another in terms of the activity of the cholinesterase standard.

To make such a cholinesterase standard, it is necessary to obtain a stable preparation which can be stored for reasonable periods of time under conditions available to most laboratories. The factor of cost would militate against the use of lyophilized preparation every time a comparative study of activity was desired. Filter paper had been used for collecting blood specimens for cholinesterase determinations (*3*). However, quantitative recovery of the red blood cell cholinesterase was not obtained by the authors upon application of the methods outlined [Ainsworth and others (*1*, *4*)]. Nevertheless the use of filter paper would allow many identical unit sources of enzyme activity to be made from small aliquots of a highly active source of cholinesterase commercially available in the form of a purified red blood cell cholinesterase. Because so many unit filter paper sources could be made from a few milliliters of the original enzyme solution, they would be relatively cheap.

This report describes the preparation of such standards, which have been stable for as long as 6 months, and gives detailed directions by means of which these unit sources of cholinesterase activity can be applied to four of the most commonly used procedures for determining the activity of this enzyme.

EXPERIMENTAL

Materials and Equipment. COLORIMETRIC METHOD. Phosphate buffer, 0.134*M*, pH 7.2, was prepared by mixing 7 parts by volume of a solution of 23.8 grams of disodium hydrogen phosphate dihydrate per liter and 3 parts of a solution of 18.2 grams of potassium dihydrogen phosphate per liter, pH being adjusted to 7.2 if necessary.

Acetylcholine, 0.04*M*, 0.7266 gram of acetylcholine chloride (Merck) in 100 ml. of 0.001*M* acetate buffer, pH 4.5: stable indefinitely in the cold.

Acetylcholine, 0.004*M*, 0.04*M* solution diluted with 9 volumes of phosphate buffer. Made freshly in quantity required for the analyses.

Hydroxylamine hydrochloride 2*M*, 27.8 grams dissolved in distilled water to 200 ml.

Above solutions are kept refrigerated when not in use.

Sodium hydroxide, 3.5*M*, 28 grams dissolved in distilled water to 200 ml.

Alkaline hydroxylamine, equal volumes of 2*M* hydroxylamine hydrochloride and 3.5*M* sodium hydroxide mixed shortly before use in a quantity required for the samples being analyzed. Made up freshly for each set of samples run.

Hydrochloric acid, concentrated acid (specific gravity, 1.18) diluted with 2 volumes of water.

Ferric chloride, 0.37*M*, 10 grams of ferric chloride hexahydrate dissolved to 100 ml. in 0.1*M* hydrochloric acid.

Potassium chloride, 0.3*M*, 22.4 grams of potassium chloride dissolved in 1 liter of distilled water.

Klett-Summerson photoelectric colorimeter with green filter No. 54.

Water bath, thermostatically controlled at 25° C.

ELECTROMETRIC METHOD. Michel's red cell buffer (*8*), 0.02*M* sodium barbital (4.1236 grams), 0.004*M* potassium orthophosphate (monobasic) (0.5446 gram), and 0.60*M* potassium chloride (44.730 grams).

For 1 liter of buffer, the reagents are dissolved in 900 ml. of distilled water; 28.0 ml. of 0.1*M* hydrochloric acid are added while shaking the solution, and the volume is then made to mark. The pH should be 8.10 at 25° C.

Acetylcholine, 0.11*M*, 2.00 grams of acetylcholine chloride in 100 ml. of distilled water.

A few drops of toluene are added to the above solutions as a preservative, and they are kept refrigerated when not in use.

Beckman pH meter Model G.

Water bath, thermostatically controlled at 25° C.

MANOMETRIC METHOD. Bicarbonate buffer, 0.025*M* containing 0.03*M* magnesium chloride. Dissolve 2.1 grams of sodium bicarbonate in 500 ml. of water. Add 6.1 grams of magnesium chloride hexahydrate. Make to 1 liter with additional distilled water. Equilibrate with a rapid stream of 5% carbon dioxide in nitrogen for 10 minutes, and keep stoppered when not in use.

Acetylcholine 0.11*M* (see electrometric method).

Warburg manometric apparatus.

TITRIMETRIC (CONSTANT pH) METHOD. Potassium chloride, 0.6*M*, dissolve 44.8 grams of potassium chloride in 1 liter of distilled water.

Sodium barbital, 0.02*M*, dissolve 4.1236 grams in 800 ml. of distilled water. Add 0.1*N* hydrochloric acid to pH 7.4; then make to 1 liter with additional water.

Acetylcholine, 0.11*M*. (See electrometric method.)

Sodium hydroxide, standardized, approximately 0.02*N*.

Beckman, Model G pH meter with shielded glass electrode.

Magnetic stirrer.

Titration vessel, 8- to 10-ml. capacity, thermostated at 25° C. The pH meter, stirrer, and titration vessel should be grounded (to minimize electrical disturbances of the glass electrode).

Microburet, graduated in 0.001 ml.

In addition, each of the above methods requires purified bovine red cell cholinesterase (Winthrop-Stearns) obtained as a nearly white lyophilized powder readily soluble in aqueous solution and general laboratory equipment and other chemicals as indicated in the text.

Procedure and Results. PREPARATION OF FILTER PAPER

Table I. Effect of Stabilization Medium on Initial Recovery of Enzyme Activity from Disks

Stabilization Medium	Concentration of Enzyme Preparation, %	Assay Method	% of Wet Activity Recovered
0.3 *M* KCl + 0.1% gelatin	1	Titrimetric	29
	1	Electrometric	25
Std. M.[a] + 0.5% gelatin	1	Titrimetric	92
	1	Electrometric	82
	0.2	Manometric	81
Std. M. + 1% gelatin	1	Electrometric	80
Std. M. + 2% bovine albumin	0.2	Manometric	81
Std. M. + 4% bovine albumin	1	Electrometric	92
	0.2	Manometric ←	91
Std. M. + 8% bovine albumin	0.2	Manometric	32

[a] Std. M. (Standard medium): 0.3*M* potassium chloride, 0.5% bovine hemoglobin, 0.008*M* phosphate buffer; adjusted to pH 7.4 after addition of stabilizer component and before addition of enzyme preparation.

STANDARDS. A stabilizing medium is first prepared containing 0.3*M* potassium chloride, 0.5% bovine hemoglobin, 4% bovine albumin, and 0.008*M* phosphate buffer. The pH is adjusted to 7.4. Then the purified bovine red blood cell cholinesterase is added to the stabilizing medium to make a 1% solution of enzyme for use in the colorimetric, constant pH, and electrometric method; and a 0.2% solution of enzyme for the manometric method. Aliquots of 0.02 ml. of the freshly made enzyme solution are placed on Whatman No. 31 filter paper with a microburet. The area occupied by the enzyme solution is delineated by the hemoglobin present in the stabilizing medium. After drying for 20 to 30 minutes, the filter paper standards (disks) are cut out and stored over desiccant in the cold.

ELUTION. For the colorimetric, electrometric, and titrimetric methods. A disk prepared with the 1% enzyme solution is placed in a 25-ml. Erlenmeyer flask and covered with 5 ml. of a leaching solution (the composition of which is given in each of the assay methods above). The flask is rotated to wet the disk and then placed in the refrigerator. Maximal elution is obtained within 1 hour.

For the manometric method. No separate elution is necessary. Maximal elution is obtained within the 15-minute period required for temperature equilibration and gassing. (The procedure is briefly outlined below.)

METHODS FOR DETERMINING ACTIVITY OF DISK CHOLINESTERASE STANDARDS. After elution cholinesterase activity in the eluate may be measured by any of the commonly employed procedures. To illustrate assay procedures applicable to measuring the activity in the eluates of the disks, brief descriptions of four commonly used methods are given.

Colorimetric Method. Each disk is eluted in 5 ml. of 0.3*M* potassium chloride. One-milliliter aliquots of eluate are then incubated with 1 ml. of 0.004*M* acetylcholine-phosphate solution for 10 minutes at 25° C. The residual acetylcholine is determined colorimetrically by the Hestrin (*7*) procedure. The quantity of acetylcholine hydrolyzed is obtained by subtracting the residual quantity measured from the 4 micromoles originally added, since nonenzymatic hydrolysis is nil under these conditions. Details have been described (*5*).

Electrometric Method (*8*). Each disk is eluted in 5 ml. of a solution of 1 volume of Michel's red cell buffer diluted with an equal volume of water, followed by removal of 2-ml. aliquots of eluate. At known intervals, 0.2 ml. of 0.11*M* acetylcholine is added with mixing and the initial pH is read 1 minute later with the Beckman pH meter. The vessels containing the mixture of eluted enzyme and substrate are incubated at 25° C. followed by reading of the pH value exactly 60 minutes after the initial pH was read. Readings are corrected for nonenzymatic hydrolysis.

Titrimetric (Constant pH) Method (*6*). Each disk is eluted in 5 ml. of 0.6*M* potassium chloride–0.1% gelatin solution at pH 7.4. A 2-ml. aliquot of eluate is transferred to the titration vessel and is followed by 1.5 ml. of additional 0.6*M* potassium chloride–0.1% gelatin solution and 1.0 ml. of 0.02*M* sodium barbital. The pH of the mixture is adjusted to pH 7.4 and 0.5 ml. of 0.11*M* acetylcholine is added with mechanical stirring. Small volumes of standard sodium hydroxide are then added at frequent intervals so as to maintain the pH at 7.4. The volume of sodium hydroxide and the time at which the galvanometer needle of the pH meter registers null at pH 7.4 are recorded. The slope of the plot relating volume of a standard alkali added to time expresses the enzyme activity and may be recalculated in terms of micromoles of alkali added per minute, a figure numerically equal to the micromoles of acetylcholine hydrolyzed per minute.

Manometric Method (*2*). A disk prepared with the 0.2% enzyme is cut into several thin ribbons which are placed in the main section of a Warburg vessel and covered with 2 ml. of 0.025*M* bicarbonate buffer, pH 7.4, containing 0.03*M* magnesium chloride. The side arms receive 0.2 ml. of 0.11*M* acetylcholine. The vessels are attached to their manometers, which are then transferred to the water bath at 25° and gassed with 5% carbon dioxide–95% nitrogen for 15 minutes while shaking. The remainder of the procedure is carried out in the usual manner. The carbon dioxide output in microliters was plotted against time, and found to be linear throughout the 60-minute interval studied.

EFFECT OF STABILIZATION MEDIUM ON RECOVERY OF ENZYME ACTIVITY OF DISKS. The effect of different stabilizing media used for the preparation of the disks on the subsequent recovery of activity was measured by making the enzyme stock solution in the various media shown in Table I. The activity of suitable aliquots along with controls of the enzyme solution was then determined by the methods indicated in the table.

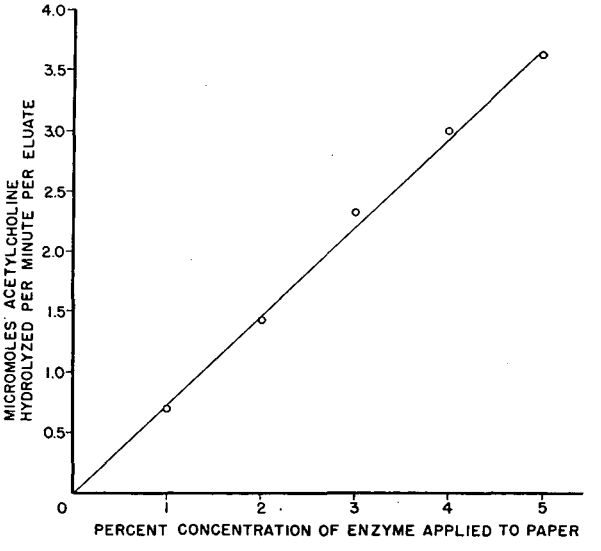

Figure 1. Relation between cholinesterase activity and concentration of enzyme on disks

Media buffered in the pH range of optimal stability of the enzyme lead to increased recovery from the dried disks. Increasing the protein content of the stabilizer up to a certain optimum also serves to increase the recovery from the disks. Above this optimum, as in the instance of the increase in bovine albumin from 4 to 8%, there is a precipitous decrease in the recovery, which is probably due to the slower drying of the heavy pellicle deposited by the enzyme in the 8% albumin leading to possible partial denaturation.

RELATION BETWEEN ENZYME ACTIVITY AND QUANTITY OF ENZYME ON DISKS. The relation between cholinesterase activity and the quantity of enzyme on the disks was studied by dissolving the lyophilized preparation in the stabilizing medium containing 0.5% gelatin so as to make a 5% stock solution. Portions of the 5% preparation were then further diluted with additional stabilizer to yield 1, 2, 3, and 4% solutions. Disks were made, and eluted in 5 ml. of potassium chloride–gelatin solution at pH 7.4 followed by determination of the activity of a suitable aliquot of the eluate by the titrimetric method. Results are shown in Figure 1. The activity recovered from the dried disks is proportional to the amount of the enzyme solution originally placed on the disks.

Table II. Reproducibility of Methods for Determining Cholinesterase Activity of Disk Standards

Disk	Colorimetric	Electrometric	Titrimetric	Manometric
	Acetylcholine hydrolyzed, micromoles	(ΔpH), (ΔpH/hr.)	Rate of addition of 0.01755M sodium hydroxide, ml./min.	Carbon dioxide output in 30 min., μl.
1	1.44	1.43	0.0178	79
2	1.49	1.42	0.0182	81
3	1.49	1.41	0.0181	78
4	1.40	1.44	0.0178	87
5	1.45	1.40	0.0186	78
6	1.42	1.42	0.0186	79
7		1.39		81
8		1.43		82
9		1.40		85
Mean	1.45	1.42	0.0182	81.0
S.D.	±0.034	±0.017	0.00036	± 3.10
% variation	2.3	1.2	2.0	3.6

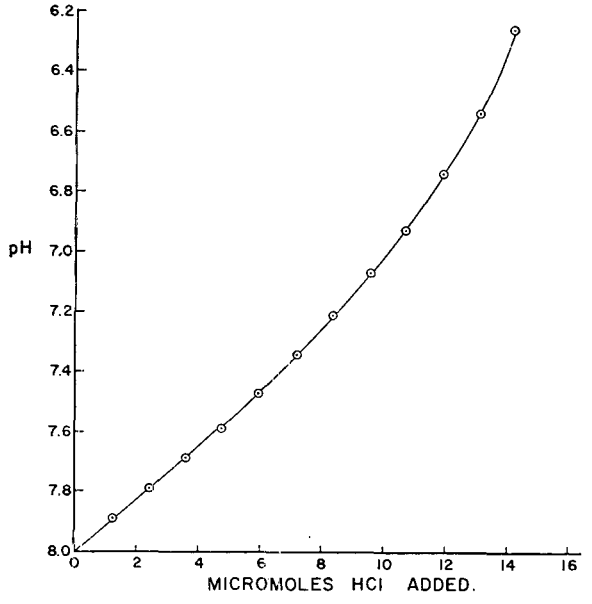

Figure 2. Titration curve of Michel's red cell buffer

Applicability of Disk Standards to Various Methods Used for Measuring Cholinesterase Activity. The applicability of the disk standards to various methods used for measuring cholinesterase activity was established by analyzing six or more disks prepared from a single source by each of the methods described, at 25° C. The activities measured in the units peculiar to each method are given in Table II. The reproducibility obtainable by each method is also presented.

Calculation of Specific Activity for Each Method of Determining Cholinesterase Activity of Disks. In order to have some measure of comparison for the methods described in the previous section, the activity measured by each method in units peculiar to it must be converted into a common denominator of measurement. The unit of activity chosen (specific activity) has been micromoles of acetylcholine hydrolyzed per minute per milligram of enzyme source. The manner of converting the activities measured by each method is given below. The general formula used is:

$$U, \text{ specific activity} = \frac{A}{t} \times \frac{1}{m}$$

where

A = activity of total eluate from 1 disk
t = time in minutes used for the measurement of enzymatic activity
m = weight in milligrams of enzyme preparation applied to the disk

Colorimetric Method. One fifth of the eluate hydrolyzed 1.45 micromoles in 10 minutes.

Therefore

$A = 1.45 \times 5$
$t = 10$
$m = 0.02$ ml. of a 1% solution $= 0.2$ mg.

Substituting:

$$U = \frac{7.25}{10} \times \frac{1}{0.2} = 3.63 \text{ micromoles/minute/mg.}$$

Electrometric Method. The results obtained in this method, which are in terms of ΔpH units, may be converted to micromoles of acetylcholine hydrolyzed by titrating Michel's red cell buffer with a standard acid, noting the successive changes in pH produced during the titration by given increments of acid. The data may be plotted on a curve as shown in Figure 2 and the micromoles of acetylcholine hydrolyzed corresponding to a given change of pH may be read off the curve.

According to the results of Table II, 2 ml. of a 5-ml. eluate produced a ΔpH change of 1.42 pH units per hour equivalent to 12.8 micromoles of acetylcholine hydrolyzed (Figure 2).

Therefore:

$A = 12.8 \times 2.5$
$t = 60$ minutes
$m = 0.2$ mg.

Substituting:

$$U = \frac{12.8 \times 2.5}{60} \times \frac{1}{0.2} = 2.67 \text{ micromoles/minute/mg.}$$

Titrimetric Method. Two milliliters of a 5-ml. eluate required 0.0182 ml. of 0.01755M sodium hydroxide per minute (see Table II).

0.0182 ml. of 0.01755M sodium hydroxide = 0.319 micromole

Therefore:

$A = 0.139 \times 2.5$
$t = 1$
$m = 0.2$ mg.

Substituting:

$$U = \frac{0.319 \times 2.5}{1} \times \frac{1}{0.2} = 3.99 \text{ micromoles/minute/mg.}$$

Manometric Method. Mean activity (Table II), 81 microliters of carbon dioxide:

$A = \frac{81}{22.4}$ micromoles of acetylcholine hydrolyzed in 30 minutes
$t = 30$

Table III. Specific Activity of Disk Standards by Various Methods of Analysis

Method	No. of Disks	Specific Activity, Micromoles Acetylcholine Hydrolyzed/Min./Mg.	S.D.
Colorimetric	6	3.63	±0.08
Electrometric	9	2.67	±0.03
Titrimetric	6	3.99	±0.08
Manometric	9	3.01	±0.11

Table IV. Effect of Storage Conditions and Stabilization Media upon Retention of Initial Activity of Disk Standards

Stabilization Media	Time of Storage	Temperature, ° C.	Relative Humidity, %	% of Initial Activity
Std. M.[a] + 4% bovine albumin	22 days	3	0[b]	100
	22 days	25	0[b]	94
	20 days	25	50	87
	22 days	25	100	62
	6 months	3	0[b]	99
Std. M. + 0.5% gelatin	6 months	3	0[b]	78
Std. M. + 1% gelatin	6 months	3	0[b]	75

[a] Std. M. (standard medium): 0.3M potassium chloride, 0.5% bovine hemoglobin, 0.008M phosphate buffer; adjusted to pH 7.4 after addition of stabilizer component and before addition of enzyme preparation.
[b] Stored over anhydrous calcium chloride.

m = 0.02 ml. of a 0.2% solution = 0.04 mg.
Substituting:

$$U = \frac{81}{22.4} \times \frac{1}{30} \times \frac{1}{0.04} = 3.01 \text{ micromoles/minute/mg.}$$

Table III summarizes the specific activities as calculated for each of the methods above together with the standard deviation to be expected for each method of assay. The variability found for each method as calculated from the ratio of the standard deviations to the mean specific activity is within a few per cent in every instance.

STABILITY OF CHOLINESTERASE DISKS UPON STORAGE. The stability of the disks prepared in the various media was studied by determining the activity of freshly made disks by one or more of the methods previously described; then storing similar disks under the conditions and for the time noted in Table IV followed by a determination of their activity.

Inspection of Table IV shows that optimal retention of initial activity is achieved by storing in the cold over a desiccant. Disks decrease to 62% of their initial activity in 22 days when exposed to an atmosphere of 100% humidity at room temperature.

The effect of the stabilization medium is shown by a comparison of the disks prepared in the bovine albumin with those prepared in the gelatin medium and stored under identical conditions. The disks prepared in the 4% bovine albumin had lost virtually no activity in 6 months, whereas those prepared in the gelatin medium had lost one fourth of their initial activity.

DISCUSSION

The conditions used in the four methods for measuring the cholinesterase activity of the disks differ in salt concentration, presence of protein stabilizer, substrate concentration, and period of time used in measurement. Because these factors all affect the stability and activity of the enzyme, it is to be expected that the specific cholinesterase activity of a given preparation, measured by different procedures but expressed in the same units, will vary from one method to the next. However, the variability of 2 to 3% found for the activity of the disks by any of the procedures described here permits their use in calibrating the activity measured by one method in terms of another, provided that a given set of conditions is consistently followed. In addition, the disks may serve to point out sources of experimental variations where these are significantly greater than the 2 to 3% variation attributable to the disks themselves.

As the degree of purity of the original enzyme preparation ultimately determines the activity of the disks, preparations other than the one used in this study will yield different specific activities. Standardization of different techniques may nevertheless be achieved with any such preparation.

Under the conditions specified in this report the titrimetric procedure gave the highest specific activity, followed in turn by the colorimetric, manometric, and electrometric procedures. The last named method has the advantage of requiring the fewest reagents, and permitting many samples to be measured with a minimum of manual operations, thus minimizing the tediousness involved in the careful performance of the titrimetric procedure when the latter is performed manually.

The colorimetric method, while requiring more reagents than the other procedures, is the fastest, and compares favorably in precision and sensitivity, with any of the methods studied. If eluates of filter paper standards are used for inhibition studies at inhibitor concentrations yielding a low level of cholinesterase activity, then the colorimetric method offers the additional advantage of determining the amount of acetylcholine remaining after exposure to the residual enzyme in the eluate, rather than the small amount of acetic acid formed in excess of the blank determination.

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Determination of Oxygen in Titanium and Titanium Alloys

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A new method is proposed for the determination of oxygen in titanium and titanium alloys. The sample is mixed with carbon and is treated with bromine at 825° C. in a 96% silica tube, using helium as a carrier gas. The oxygen in the sample combines with the carbon to form carbon monoxide, which is then oxidized to carbon dioxide with hot copper oxide. The carbon dioxide is absorbed into a weighed bulb containing Ascarite. The bromine and titanium tetrabromide are removed by the use of a dry ice and water bath followed by two dry ice and alcohol baths. To remove the last traces of bromine compounds, the gases are passed through granular zinc heated to 350° C. The method was applied to the determination of oxygen in commercial titanium and titanium alloys. This covers the range of about 0.05 to 0.5% oxygen.

THE determination of oxygen in titanium and titanium alloys is important, as oxygen has a marked effect on titanium and titanium alloys, causing loss of ductility, increased hardness, and grain refinement.

The amount of oxygen found in titanium and titanium alloys varies over wide limits. Iodide titanium—i.e., titanium formed by the pyrolysis of titanium iodide—contains about 0.005 to 0.02% oxygen, while sponge titanium and "commercially pure" titanium contain about 0.05 to 0.15% oxygen. Commercial titanium alloys contain about 0.1 to 0.3% oxygen, although alloys containing up to 11% oxygen have been prepared. Oxygen is dissolved in the titanium lattice with metallic bonding, a TiO phase being produced (*21*, *33*). Oxygen is not found in titanium in pinholes as the gas or as oxide occlusions, because of the great affinity of oxygen for titanium. An extremely thin layer of titanium oxide is found on titanium and titanium alloys that have

been exposed to the atmosphere (*14*). The thickness of the oxide layer is much increased if the metal has been oxidized by heating in the air—for instance, in heat treatment processes.

PREVIOUS METHODS

Oxygen has been determined in titanium and titanium alloys by vacuum fusion methods (*1, 2, 8, 37, 39, 42, 44*). In these methods the metal is fused in the presence of carbon, the oxygen forming carbon monoxide which is subsequently oxidized to carbon dioxide. The vacuum fusion method requires costly apparatus and trained personnel. Difficulty is sometimes encountered when alloying elements, such as manganese, are present which volatilize on fusion of the sample, condense in another part of the apparatus, and recombine with the oxygen.

Chlorination techniques have been used for the determination of oxygen in titanium, the titanium being volatilized as titanium tetrachloride by passing dry chlorine over the sample at 400° C. (*2, 6*). The oxygen remains as titanium dioxide (TiO_2) and is calculated by determining the titanium colorimetrically. A correction must be made for the carbon present in the sample (*6*), and the chlorination reaction must be carefully controlled to prevent attack on the oxide by the chlorine with the production of gaseous oxygen (*16*). If molybdenum, tungsten, or vanadium is present, some oxygen may be lost as the oxychloride (*7, 31, 41*). Instead of chlorine, dry hydrogen chloride can be used (*2, 27*).

Oxygen has been determined in titanium (not titanium alloys) by making use of the fact that the hardening effect of oxygen and nitrogen is additive. Knowing the Brinell hardness and the nitrogen content, it is possible to determine the oxygen content (*39*). Oxygen in titanium has also been determined by radioactivation (*2*). The titanium specimens are subjected to a fast neutron bombardment for 30 seconds, followed by the observation of the activity after a known time interval. The method is based upon the detection of approximately 6.1 to 7.1 m.e.v. gamma activities with a half-life, $T_{1/2} = 7.35$ seconds, resulting from the $O^{16}(n,p)N^{16}$ reaction. The method requires a calibrated series of titanium specimens of known oxygen content. Another means of determining oxygen in titanium is by mass spectrometer techniques (*2, 25*). A known amount of oxygen-18 tracer is added to the sample. The oxygen is extracted as carbon monoxide by vacuum fusion and the ratio of oxygen-18 to oxygen-16 is determined by the mass spectrometer. The oxygen content of the original metal is then readily calculated. Attempts have been made to determine oxygen in titanium spectrographically by making use of the fact that titanium oxide (TiO) spectral bands are emitted when titanium is sparked in a vacuum (*2, 45*). So far the method has proved of only limited value (*45*).

Oxygen in oxygen-titanium alloys, containing rather large amounts of oxygen and only insignificant amounts of elements other than oxygen and titanium, can be determined by difference after oxidizing the titanium to titanium dioxide by igniting in oxygen (*17, 22*) or after determining the titanium present by volumetric means (*5*). Jenkins and Worner (*22*) found the oxygen content of a series of titanium-oxygen alloys, prepared by heating titanium in oxygen, by taking the gain in weight to be oxygen. Hickman and Gulbransen (*18*) studied the oxide films produced on titanium and titanium alloys at 300° to 700° C. by means of electron diffraction techniques.

Suggestions have been made that oxygen in titanium might be determined by using fluorine or nonaqueous hydrogen fluoride (*2*). By the use of fluorine at elevated temperatures the oxygen would probably be converted to gaseous oxygen (*2, 24*), and by the use of hydrogen fluoride at elevated temperatures, the oxygen would probably be converted to water (*2*). It is known that fluorine attacks titanium at 150° C. (*34*). Nonaqueous hydrogen fluoride attacks titanium only very slightly at room temperature (*20*). It reacts slowly with titanium oxide at 550° C., but volatilization is not complete after 2 hours (*32*). Apparatus for fluorination techniques would have to be constructed of special materials, possibly nickel. A disadvantage of the use of fluorine or hydrogen fluoride is that commercial fluorine always contains some oxygen, and commercial nonaqueous hydrogen fluoride always contains some water. A method has been described for the determination of oxygen in titanium oxide and other oxides by the use of bromine trifluoride (*19*). According to preliminary work the method is applicable to the determination of oxygen in titanium metal (*41*). However, certain alloying elements in titanium alloys such as molybdenum offer difficulty because of the stability of the oxyfluorides (*19, 41*).

Oxygen cannot be determined in titanium by reduction with hydrogen as is done with steels, iron powder, bismuth, and copper (*11, 30, 40*). On treatment with hydrogen, the titanium absorbs hydrogen, and the oxides present are merely reduced to lower oxides (*12*). Reduction with carbon at 1200° C. in an atmosphere of nitrogen to produce carbon monoxide has been used for the determination of oxygen in steels (*36*). Such a procedure is not applicable to titanium because at 1200° C. the oxides of titanium are merely reduced to lower oxides by carbon (*16*). Solution techniques and electrolytic techniques (frequently applied to steels), whereby the metal is dissolved and the oxide remains (*4*), are not applicable to titanium. Oxides present in titanium dissolve almost as readily as the metal (*10, 15*). Amalgamation techniques applied to the determination of oxygen in sodium (*29*) and lead (*35*), whereby the metal is amalgamated and the oxide remains unattacked, are not applicable to titanium because titanium does not amalgamate. Oxygen has been determined in steels by melting with metallic aluminum and then determining the aluminum oxide formed (*30*). Such a method is not applicable to titanium, because titanium has almost as great an affinity for oxygen as aluminum (*38*). When aluminum is melted with a titanium alloy containing oxygen, an alloy of titanium, oxygen, and aluminum is formed, but the oxygen is still tied to the titanium.

BROMINATION–CARBON REDUCTION METHOD

An entirely new method is proposed for the determination of oxygen in titanium and titanium alloys based upon a reaction previously used (*3, 28*) for the preparation of titanium tetrabromide by heating a mixture of titanium dioxide and carbon with bromine:

$$TiO_2 + 2C + 2Br_2 \rightarrow TiBr_4 + 2CO$$

In applying the reaction to titanium and titanium alloys, the sample was mixed with carbon in a platinum, gold, quartz, or 96% silica boat and the mixture treated with bromine in a 96% silica tube at 825° C. while helium was passed through the system. The resultant carbon monoxide was oxidized to carbon dioxide by copper oxide at 500° C. and the carbon dioxide absorbed into a weighed bulb containing Ascarite. An important problem in perfecting the method was to find means for removing the titanium tetrabromide and bromine. It was found that these substances could be frozen out by the use of a dry ice and water bath followed by two dry ice and alcohol baths. To remove the last traces of bromine compounds, the gases were passed through granular zinc heated to 350° C. or compacted sponge silver heated to 600° C. The zinc and silver were equally effective, but zinc is preferable because it is cheaper. The use of zinc in place of silver was suggested to the authors by Kallmann (*23*).

Before passing through the zinc, the gases were passed through Ascarite and Anhydrone to remove acidic impurities. The use of Ascarite to remove impurities from carbon monoxide has been recommended by Dinerstein and Klipp in organic analysis (*9*). After passing through the copper oxide, the gases were passed through Anhydrone to remove water that would be formed by the oxidation of hydrogen. Two gas-washing bottles containing sulfuric acid were included in the system in order to observe the bubble rate. Two safety traps were also included, one before the bottle containing the bromine, the other before the final sulfuric

acid trap. The helium was purified by passing it through copper and copper oxide heated to 500° C., and then through Ascarite and Anhydrone. This treatment removed such impurities as oxygen, hydrogen, carbon monoxide, carbon dioxide, hydrocarbons, sulfur dioxide, and water. A mercury blow-off valve was placed after the tank of helium. Traces of water in the bromine were removed by the addition of sulfuric acid.

SIGNIFICANCE OF THE BLANK

An hourly blank must be deducted. This blank is obtained by passing bromine over 1.5 grams of graphite for 3 hours. The average blank was found to be about 1.0 mg. of carbon dioxide per hour when a 96% silica or quartz boat was used, 0.7 when a platinum boat was used, and 0.4 when a gold boat was used. The average run takes 2 hours. The higher blanks obtained with 96% silica or quartz boats seemed to be due to attack of the boats, as indicated by the fact that they became dull on use. The difference in blank rates when platinum and gold boats were used was extremely perplexing until it was traced to the fact that platinum develops an oxide coating, especially on heating (*26*, *43*). Kubaschewski and Hopkins state (*26*), "With the exception of gold no metal including platinum is stable in air. Although platinum is considered to be a precious metal, its affinity for oxygen is appreciable. The bulk of the metal is not easily converted into oxides but a strongly adherent absorbed layer of oxide which is very difficult to remove is formed during exposure to air. Treatment of normal platinum powder with dry hydrogen even above 1200° C. removes absorbed oxygen only very slowly." Confirmatory evidence that platinum develops an oxide coating is the loss in weight of platinum when it is heated in air or oxygen above 1200° C. through the formation of volatile platinum oxides (*26*). If platinum is heated in nitrogen or argon, this loss in weight does not occur. The blanks obtained with platinum boats were the same, whether the boats were dried by heating in an oven at 200° C., in a muffle at 1200° C., or in a flame. The blanks obtained with platinum boats did not decrease if the boats were cleaned by a carbonate or bisulfate fusion. Spectroscopic examination failed to show any impurities in the platinum or gold boats that might constitute a source of the blank.

No evidence of the formation of either platinum carbide or gold carbide by reaction between the graphite and the boats was ever noted. Platinum carbide can be formed from platinum and graphite only if graphite is added to molten platinum. The one gold carbide known, Au_2C_2, can be formed only by passing acetylene into aqueous gold solutions. Platinum was found to be unattacked by bromine, while gold was only very slightly attacked. According to some investigators, gold is rapidly attacked by bromine. This discrepancy is due to the fact that water and oxygen are catalysts for the attack of gold by bromine. If dry, oxygen-free bromine is used, the attack of gold will be very slight. Satisfactory results were obtained with both platinum and gold boats. However, because lower blanks were obtained with gold boats, it is recommended that the boats be made of this material.

When no sulfuric acid was added to the bromine, a very high blank was obtained (about 4.6 mg. of carbon dioxide per hour), indicating that some water was present in the bromine, and would react with the hot graphite to give carbon monoxide. The relationship between water and bromine is complicated, the following reaction taking place:

$$Br_2 + H_2O \rightleftarrows HBrO + HBr$$

The equilibrium of the above reaction is to the left, and in the presence of excess hydrogen ion the equilibrium would be expected to be shifted even further to the left (*34*). Therefore, after the addition of the sulfuric acid to the bromine, the amount of hypobromous acid left in solution might be small, especially as the sulfuric acid would take up the water. The hypobromous acid left in solution would partially decompose according to the following equations (*34*):

$$4HBrO \rightarrow O_2 + 2H_2O + 2Br_2$$

$$5HBrO \rightarrow HBrO_3 + 2H_2O + 2Br_2$$

All of the oxygen formed by the decomposition of the hypobromous acid and possibly some of the hypobromous acid itself would be driven off in the flushing of the bromine bottle with helium following the addition of sulfuric acid. Bromic acid is not significantly volatile. It is conceivable that the sulfuric acid removes volatile organic impurities frequently present in bromine by dissolving them or reacting with them (sulfonation). When the sulfuric acid is added to the bromine, two layers are formed, with the sulfuric acid on top. The solubility of bromine in sulfuric acid is about 0.75%. The solubility of sulfuric acid in bromine is not precisely known. The solubility of water in bromine is somewhat less than 0.1%. Reliable figures are not available because of the difficulty of determining water in bromine.

The slight volatilization of sulfuric acid was found to account for a small part of the blank in the method (on the average about 0.05 mg. an hour) because of the following reaction:

$$C + H_2SO_4 \rightarrow CO + SO_2 + H_2O$$

The effect of the sulfuric acid was tested by adding 200 ml. of sulfuric acid alone without bromine to the 1-liter bromine bottle, and flushing with helium for 2 hours (a long flushing period is needed because it takes some time to drive out all the air from a 1-liter bottle, if only a small volume of liquid is present). The helium from the bottle was then passed over hot graphite as described. The effect of the sulfuric acid is due to the vapor pressure of sulfuric acid. No sulfur trioxide would be volatilized from the reagent grade 96% sulfuric acid, but, if sulfuric acid of greater than 98% strength were used, some sulfur trioxide would volatilize. Unsuccessful experiments were carried out on other dehydrating agents for removing water from the bromine. The use of a tube or tower of Anhydrone as a substitute for (or in conjunction with) the sulfuric acid gave very erratic results for some unknown reason.

Another source of the blank could be oxygen in the graphite. The National Carbon Co. AGR grade graphite used contains traces of oxygen gas, water vapor, and possibly metal oxides. When graphite containing oxygen gas is heated, the oxygen is driven off mostly as carbon dioxide and carbon monoxide rather than as oxygen. The graphite used gave an ash content on ignition in air of 0.05%. Spectroscopic examination showed the presence of silicon, aluminum, boron, iron, magnesium, vanadium, and sodium. Because of the extremely high temperature used in the manufacture of graphite, the impurities were probably present as carbides rather than as oxides. Spectrographic graphite containing less than 0.002% metallic impurities was found to give a blank of 0.3 mg. with a gold boat. The size of the blank in the method increased with increasing amounts of graphite—when 3 grams of AGR graphite was used rather than the recommended 1.5 grams an average blank of 1.0 mg. was obtained with a platinum boat.

Consideration was given to the possibility that the glass of the reaction tube might constitute a source of the blank. When glass is heated, water vapor from two sources is released—that absorbed on the glass and that obtained from inside the glass. In the latter case the water is not present in the glass as such but is produced by a combination of hydrogen and hydroxyl groups that are attached to the glass molecule. As constant weight was readily obtained at the operating temperature of 825° C. on passing helium over the graphite without bromine, it would seem that the glass is not a prime source of the blank. However, if too high an operating temperature is used (above about 910° C.) or if the heating of the reaction tube is uneven, difficulty is sometimes

encountered in obtaining constant weight, and higher blanks may be obtained. The glass might be the source of this trouble. The use of a quartz reaction tube instead of a 96% silica reaction tube did not permit the use of a higher operating temperature. The blank rate increased somewhat with increasing flow of helium. The recommended flow of helium is 110 cc. per minute.

The method described in this paper is recommended for the determination of oxygen in commercial titanium and titanium alloys. This covers the range of about 0.05 to 0.5% oxygen.

To convert carbon dioxide to oxygen, the figure 0.3636 is used, because one atom of oxygen in the carbon dioxide is derived from the copper oxide.

REAGENTS

Copper(II) oxide, wire form, analytical reagent.

Granular blister copper (Ledoux and Co., Teaneck, N. J.). This has a copper content of about 99.5%.

Zinc, granular, 20-mesh, C.P.

Ammonium hydroxide, specific gravity 0.90.

Anhydrone.

Ascarite.

Carborundum, 600-mesh (Arthur H. Thomas Co., Philadelphia, Pa.).

Glass wool (Pyrex wool, Corning Glass Works, Corning, N. Y.).

Fluorolube, MG grade (Hooker Electrochemical Co., Niagara Falls, N. Y.).

Carbon tetrachloride, reagent grade.

Ethyl alcohol, 95%.

Nitric acid, specific gravity 1.41.

Hydrofluoric acid, 48%.

Graphite powder, prepared by scraping 0.75-inch graphite rods, grade AGR (National Carbon Co., Niagara Falls, N. Y.), with a file, razor blade, or sharp knife and sieving through a No. 80 sieve. It is stored in a glass-stoppered bottle. The instrument used to scrape the rods should be free from rust and grease and if necessary, cleaned with carbon tetrachloride.

Bromine, C.P., AMERICAN CHEMICAL SOCIETY specification.

Helium, grade A, about 99.8% helium. The helium used was obtained from the U. S. Army. The same grade is available commercially from several firms.

APPARATUS

A diagram of the apparatus is shown in Figure 1. The reaction tube and the electric heater for heating the reaction tube were tilted downward at a very slight angle to minimize possible backflow of certain metal bromides in the reaction tube. The use of two interchangeable heaters for heating the reaction tube eliminates the necessity of a waiting period between runs while the heater cools to room temperature. All the heaters used were constructed in the workshop of this laboratory and were designed for 115 volts. They were made by winding Nichrome wire around Sillimanite tubes, 1.25 inches in diameter, and then surrounding the Sillimanite tubes with 2.75 inches of block asbestos insulation. The heater for the reaction tube (26 in Figure 1) and the heater for the copper–copper oxide tube (No. 9) used 18-gage, 8-ohm wire (0.406 ohm per foot). These heaters drew about 10 amperes and consumed 1150 watts. The heaters for the zinc (No. 39) and for the copper oxide (No. 42) used 20-gage, 16-ohm wire (0.631 ohm per foot). These heaters drew about 8 amperes and consumed 920 watts. The heater for the reaction tube was regulated by pyrometer control. All the other heaters were controlled by Powerstats, the reading on the Powerstat corresponding to the recommended temperature being determined by standardizing with a portable thermocouple-type pyrometer. A central control board for regulating all the heaters by pyrometer control is recommended, if the necessary equipment is available.

The parts of the apparatus that contain bromine should be kept under a hood. The apparatus can be made compact by placing the heater for the reaction tube (No. 26 in Figure 1) crosswise over the heater for the copper–copper oxide tube (No. 9), by having the first Ascarite-Anhydrone tube vertical, and by doubling back after the zinc column (No. 40). All the ground-glass joints on the apparatus should be held together by springs.

PROCEDURE

Prepare the train as shown in Figure 1. Turn on all electric heaters, except those used to heat the reaction tube and the zinc. Add to the bromine bottle about 200 ml. of sulfuric acid and about 550 ml. of bromine (4 pounds). With system disconnected before the reaction tube, pass helium through the mixture of bromine and sulfuric acid for 1 hour in order to drive out any oxygen. After the flushing is complete, turn the stopcocks so that the helium bypasses the bottle containing the bromine.

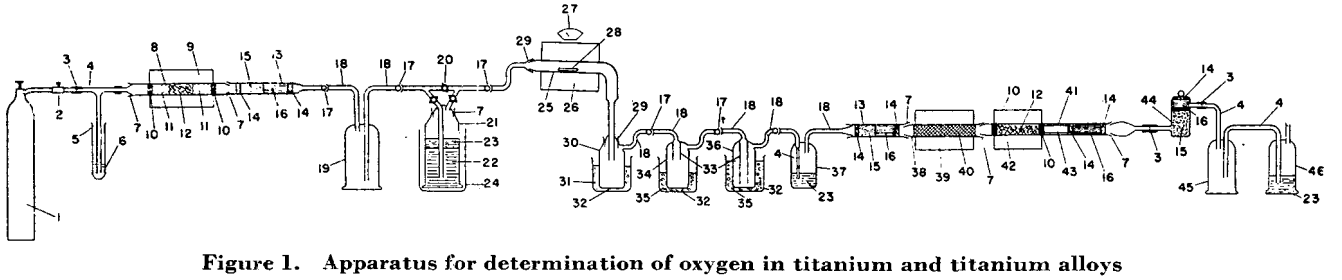

Figure 1. Apparatus for determination of oxygen in titanium and titanium alloys

1. Tank of helium
2. Needle valve
3. Gum rubber tubing, small piece
4. Borosilicate glass tubing, 7 mm.
5. Mercury blow-off valve made from borosilicate glass test tube 11.5 inches long and 0.75 inch in diameter. Inlet tube, made from 7-mm. borosilicate glass tubing, reaches to within 0.25 inch of bottom of test tube
6. Mercury
7. Ground-glass joint (29/42)
8. Copper–copper oxide tube, 96% silica, 1 inch in diameter and 16 inches long
9. 11-inch electric heater for copper–copper oxide tube, 500° C. Heating elements extend over entire length
10. Crumpled ball of fine copper wire, diameter about 0.016 inch.
11. Granular blister copper
12. Copper oxide, wire form
13. Ascarite-Anhydrone purification tube, borosilicate glass, 1 inch in diameter and 8 inches long
14. Glass wool
15. Ascarite
16. Anhydrone
17. Ball joint (18/9)
18. Borosilicate glass tubing, 10 mm.
19. Suck-back trap for bromine, capacity about 250 ml.
20. Stopcock
21. Bromine bottle, capacity about 1 liter
22. Bromine
23. Sulfuric acid
24. Steel or heavy plastic beaker to protect bromine bottle
25. Reaction tube, 96% silica or quartz. Horizontal portion is 1 inch in diameter, 15 inches long. Vertical portion is 1 inch in diameter at top, 18 mm. in diameter at bottom, 12 inches long. Distance from top of reaction tube to ice water trap is 8 inches. End of reaction tube reaches to 3 inches from bottom of dry ice and water trap. Distance between top of bromine bottle and reaction tube is 8 inches
26. Electric heater for reaction tube, 825°C., controlled by pyrometer, 10 inches long. 825° C. maintained evenly over middle 6 inches of heater
27. Pyrometer
28. Gold boat about 3.5 inches long
29. Ground-glass joint (24/40)
30. Dry ice and water trap, borosilicate glass, 1.25 inches in diameter and 6 inches in height. Inlet tube reaches to 3 inches from bottom. Side arm 2 inches in horizontal length
31. Dry ice and water
32. Insulated beaker—500- or 600-ml. beaker surrounded by glass wool and heavy paper. Thermos bottle can also be used
33. Borosilicate glass tubing, 18 mm.
34. First dry ice and alcohol trap, borosilicate glass, 1.25 inches in diameter and 6 inches in height. Inlet tube reaches to 4 inches from bottom. Both side arms 2 inches in horizontal length
35. Dry ice and alcohol
36. Second dry ice and alcohol trap, borosilicate glass, 1.25 inches in diameter and 6 inches in height. Inlet tube reaches to 0.5 inch from bottom. Both side arms 2 inches in horizontal length
37. Sulfuric acid bubble counter, borosilicate glass, 1.25 inches in diameter and 6 inches in height. Inlet tube reaches to 0.5 inch from bottom. Both side arms 2 inches in horizontal length
38. Tube for zinc, 96% silica, 1 inch in diameter and 14 inches long
39. Electric heater for zinc, 350° C. Heater is 8.5 inches long. Heating elements extend over entire length
40. Zinc, held in place by glass wool
41. Copper oxide-anhydrone tube, 96% silica, 1 inch in diameter and 20 inches long
42. Heater for copper oxide, 500° C. Heater is 7 inches long. Heating elements extend over entire length
43. 96% silica tube, 0.75 inch in diameter and 2 inches long, to separate copper oxide from anhydrone
44. Nesbitt absorption bulb
45. Small suck-back trap for sulfuric acid
46. Small sulfuric acid bubble counter

Prepare the sample in the form of chips or strips about 0.03 inch thick. Clean with carbon tetrachloride and dry thoroughly in an oven at 50° C. Weigh out 2 grams of the sample and 1.5 grams of graphite powder. Place about one half the graphite powder on the bottom of the boat, place the sample on this carbon, and cover the sample with the rest of the carbon. Push the sample to the center of the heater with a stout wire bent at the end. Assemble the system, but do not connect the Nesbitt absorption bulb. Adjust the flow of helium to a very fast rate and allow the system to flush for a few minutes. Decrease the flow of helium to 110 cc. per minute. Turn on the heaters for the reaction tube and the zinc. While the heaters are coming up to temperature, add about 300 ml. of water to the beaker for the dry ice and water trap and then add several pieces of dry ice, each about 0.75 cubic inch in volume. Add about 150 ml. of ethyl alcohol to the beaker for the first dry ice and alcohol trap and about 250 ml. for the second dry ice and alcohol trap. Add pieces of dry ice about 0.75 cubic inch in volume to the alcohol until the strong effervescence ceases. The dry ice in the beaker for the first dry ice and alcohol trap should be 1 inch below the end of the inlet tube; for the second dry ice and alcohol trap it may completely fill the beaker. Add more dry ice occasionally during the determination.

When the temperature has reached 825° C., connect the Nesbitt absorption bulb and allow the helium to pass through the system for 10 minutes. Close the stopcock of the Nesbitt absorption bulb and disconnect. Open the stopcock momentarily to the atmosphere to equalize the pressure and weigh, using an empty bulb containing lead shot as a counterpoise. Reconnect the bulb and weigh again after 10 minutes. If constant weight has not been reached, repeat the process. The bulb may lose weight when first connected, because of displacement of the air by the lighter helium.

Turn the stopcocks so that the bromine flows through the system. Continue the flow of bromine for 30 minutes after the reaction is ended, as indicated by the complete disappearance of the cloud in the vertical portion of the reaction tube. The use of a flashlight to observe the end of the reaction is convenient. Note the time required for the complete run. Turn off the bromine and switch off the heater for the reaction tube, but allow the helium to flow. Turn the stopcock of the Nesbitt absorption bulb and weigh, using the same technique as before. The gain in weight is carbon dioxide. Calculate the per cent oxygen as follows:

$$\text{Per cent oxygen} = \frac{36.36\,(W - BT')}{M}$$

where W = weight of carbon dioxide, grams
B = blank, grams of carbon dioxide per hour
T = time for run, hours
M = weight of sample, grams

The blank is found by carrying 1.5 grams of carbon through the determination. The flow of bromine is continued for 3 hours and the gain in weight is divided by 3 to obtain the hourly blank.

To prepare for the next run disconnect the reaction tube and the three traps. Cautiously pour the contents of the dry ice and water trap into a 3-liter beaker containing about 1 liter of tap water. Do not add the water to the dry ice and water trap. If this is done the contents will spatter badly because of the vigorous reaction between the titanium tetrabromide and water. Add water to the two dry ice and alcohol traps (there is no danger of spattering with these traps), shake to dissolve any frozen bromine, and pour the contents into the 3-liter beaker. Add ammonium hydroxide to the contents of the 3-liter beaker to destroy the bromine. Rinse all three traps with water and dry by heating in an oven at 200° C. Remove the boat from the reaction tube and wash the reaction tube with cleanser, using a long brush. Rinse the reaction tube well with water and dry it thoroughly by heating with a blast burner while holding it with an asbestos glove. Remove the carbon from the boat and clean the boat by rubbing with a cloth and cleanser on a small glass plate. Soak the boat for 2 hours in an acid mixture made of equal parts of nitric and hydrofluoric acids. This acid mixture is best stored in a plastic beaker. Wash the boat well with water and dry it by heating to 600° C. (the melting point of gold is 1060° C.). Allow the heater used for heating the reaction tube to cool to room temperature before proceeding with the next run or substitute another heater. Before starting the next run flush out the system before the first sulfuric acid bubbler with helium to prevent oxidation of the zinc.

Before shutting off the helium, make sure that the stopcocks on both sides of the bromine bottle are shut, to prevent possible suckback of the bromine.

NOTES ON METHOD

Leaks must be prevented as they can lead to high results if they occur before the reaction tube and to low results if they occur after the reaction tube. Leaks can be detected in parts of the apparatus conducting bromine by holding the stopper from a bottle of ammonium hydroxide close to the connections; if a leak is present, a white cloud of ammonium bromide will be observed. Ground-glass joints, ball joints, and stopcocks should be ground together with a paste made from 600-mesh Carborundum and water. Stopcocks should be greased with Fluorolube grease but too much should not be used; otherwise leaks will occur. All other greases tested were attacked by the bromine, causing high results for oxygen. Ball joints connecting the traps should be lubricated with sulfuric acid.

An all-glass apparatus is recommended. Rubber stoppers, rubber tubing, or plastic tubing can cause high and erratic results, especially if these materials come in contact with bromine or become warm. In this laboratory polyethylene, polystyrene, and vinyl resin plastic tubings were not found to be resistant to bromine.

The outlet tube for the bromine bottle and the inlet tube for the reaction tube should have a combined height of about 8 inches to prevent droplets of bromine or sulfuric acid from being carried over mechanically into the reaction tube.

Almost all of the titanium tetrabromide and a fairly large part of the bromine are removed by the dry ice and water bath. The boiling point of titanium tetrabromide is 230° C. and its freezing point is 39° C., while the boiling point of bromine is 59° C. and its freezing point is −7.2° C. The titanium tetrabromide does not solidify in the dry ice and water trap because it dissolves in the bromine. The temperature obtained with the dry ice and water bath is 0° C., as the dry ice quickly becomes coated with a layer of regular ice. A few minutes after being added to the water, the pieces of dry ice coalesce into one chunk which surrounds the cylindrical trap. A dry ice and water bath is much more convenient than a regular ice bath and requires far less attention. The temperature obtained with the dry ice and alcohol mixture was about −70° C. About the same temperature was obtained when acetone was substituted for the alcohol.

The inlet tube of the first dry ice and alcohol trap must not be less than 18 mm. in diameter and the end of the inlet tube must not be less than 4 inches from the bottom of the trap. Otherwise, the inlet tube will become clogged with frozen bromine. For the same reason, too much alcohol and dry ice should not be present in the beaker for the first dry ice and alcohol trap. There is no danger that a pocket of gas will form at the bottom of the trap, because the diffusion rate is very rapid when there is a rapid flow of gas.

Two or three successive runs on the same day may be made without cleaning the dry ice and water or dry ice and alcohol traps.

The temperature of the heater used to heat the reaction tube is moderately critical. The best temperature is about 825° C. If the temperature is much less than this, low results for oxygen will be obtained, and if the temperature is too high, the size of the blank may increase. The thermocouple should be placed in the center of the heater for the reaction tube. The heater used required 15 minutes to reach 825° C.

To purify helium, the gas is passed through a heated tube containing copper, copper oxide, and copper in that order. Copper must be placed after the copper oxide in order to absorb any oxygen released by partial decomposition of the copper oxide.

$$4CuO \longrightarrow 2Cu_2O + O_2$$

At 500° C. the pressure of the oxygen for the reaction is significant (*32*). The temperature of the copper oxide should be about 500° C. as at lower temperatures the oxidation of hydrocarbons (which may be present in helium) by copper oxide is incomplete. Granular blister copper is far superior to copper shavings for the removal of oxygen from helium. Powdered copper cannot be used, because it sinters easily and is contaminated by organic material. The authors found the oxygen content of grade A helium to be appreciable and the variation between different tanks considerable.

Glass wool should not be substituted for the crumpled balls of fine copper wire in the copper–copper oxide tube or the copper oxide tube. The partial decomposition of the glass wool on strong heating can cause erratic results.

The granular zinc should be replaced every week. The zinc should fill the entire width of the tube and no air space should be left at the top.

The Anhydrone and Ascarite contained in the tube before the zinc should be replaced weekly and should be packed firmly by compressing with a rod.

The sulfuric acid in the first bubbler should be replaced daily; recommended height of sulfuric acid in the first bubbler is 2 inches.

The only materials that could be used for boats were platinum, gold, quartz, and 96% silica glass. Boats made from porcelain, alumina, silicon carbide, mullite, tantalum, silver, nickel, and molybdenum were badly attacked. Attempts to fabricate boats by compressing such salts as calcium bromide and calcium chloride were not successful. Graphite boats gave erratic results.

The flow of helium should be regulated to about 110 cc. per minute. If the rate is much less than this the reaction is slow; if much more, less reliable results and somewhat higher blanks will be obtained. The flow rate is best measured by attaching a Mariotte bottle to the system after the copper oxide–Anhydrone tube, while the temperature of the reaction tube is coming up to 825° C. The Mariotte bottle is made by attaching near the bottom of a 2-liter bottle a piece of borosilicate glass tubing 6 inches in length and 8 mm. in width. The tubing is bent at 90° in the middle and is pulled out at its exit end so that the internal diameter of the exit end is 2.5 mm. A 500-ml. graduated cylinder is placed under the exit end. The bottle is filled with tap water and the system is connected to the bottle by means of rubber tubing and a one-holed rubber stopper. The first 100 ml. of water coming over is disregarded, and then the volume coming over in 60 seconds is measured.

The reaction tube and the inlet tube for the reaction tube must be dried thoroughly; otherwise high results will be obtained. The reaction tube should be connected within a few minutes after being heated, to avoid condensation of moisture from the air. The inlet tube should be stored in a desiccator when not in use and brushed with a flame just before it is connected. After the helium is shut off, a small Anhydrone tube with a male ball joint at its end should be connected to the exit tube from the bromine bottle to keep out air moisture. Acetone should not be used for drying the glassware, as it often leaves a film.

The use of chips or strips of the sample is recommended. If large pieces are used, the reaction is slow. The use of fine drillings will lead to high results.

Cleaning the chips with carbon tetrachloride must not be omitted, as grease on the sample will cause high results for oxygen. The carbon tetrachloride must, of course, be completely evaporated. Ether and trichloroethylene are not satisfactory solvents for the grease, as they often leave a film. Benzene can be used satisfactorily. If the original specimen is suspected of containing an oxide coating, its exterior surface should be removed by mechanical means.

Only graphite is satisfactory for the method; other forms of carbon will give high results because of their high oxygen content. The graphite cannot be used again—on treatment with bromine it becomes highly activated and on exposure to the atmosphere absorbs large amounts of oxygen. Attempts to "regenerate" the graphite by igniting in nitrogen were not successful.

The sample must be completely covered with carbon; otherwise some titanium oxide will remain unreacted. However, if the sample is covered with too much carbon, the reaction will be slow.

The heater for the reaction tube must be cooled to room temperature (by means of a blast of air) before a second run is made, or a second heater substituted. If the sample is introduced into a hot tube, absorption of oxygen by the titanium and carbon will cause high results for oxygen. Oxidation of titanium starts at about 150° to 200° C., and as the temperature is increased a thickening layer of oxide is formed (*13*, *14*).

The reaction tube and the inlet tube for the reaction tube should be cleaned after every few runs by soaking with dilute hydrofluoric acid (1 to 3) for a few minutes.

A reaction tube with a bulb at the end, in order to run large samples, did not prove feasible for titanium or titanium alloys, as fumes of the titanium tetrabromide in the bulb made it impossible to see when the reaction was finished.

The absorption bulb must be maintained at an even temperature during the determination, since the bulb gains weight when the temperature decreases and loses weight when the temperature increases. This gain and loss are due mainly to the change in density of the helium in the absorption bulb in accordance with Charles' law. In a series of experiments the change in weight of the absorption bulb from 15° to 25° C. was about 0.2 mg. per degree. The change was less when helium was used than when nitrogen or argon was used, because helium is lighter. To avoid changes in temperature, the absorption bulb must be kept away from drafts and must not be placed under the hood. Placing the absorption bulb in the balance case for 10 to 30 minutes prior to weighing so that it will assume the temperature of the balance case is not feasible for the method described. It is essential that the absorption bulb be weighed using as a counterpoise an empty absorption bulb containing lead shot to help eliminate errors due to buoyancy of the absorption bulb in air. The counterpoise must be open to the air, so that it will assume atmospheric pressure, and it is best to keep it in the balance case at all times. Placing it alongside the absorption bulb during the run did not increase the accuracy and precision of the weighings. The absorption bulb must not be wiped prior to weighing, as the static charges produced will cause erratic results.

Nothing is gained by connecting the absorption bulb in the system before the temperature of the reaction tube reaches 825° C.; time is lost in flushing out the heavier air from the absorption bulb.

In making a determination the system should be swept out for 30 minutes after the disappearance of the titanium tetrabromide fumes, because most of the reaction between the titanium oxide, carbon, and bromine takes place toward the very end of the run. If a sweep-out period much less than 30 minutes is used, low results will be obtained.

If the apparatus has been idle for three or more days, the bromine bottle should be flushed with helium for a few minutes to drive off any oxygen that may have diffused into the bottle.

If laboratory design does not permit the parts of apparatus that conduct bromine to be placed under a hood, the entire apparatus may be kept outside the hood, if the bromine bottle is flushed so that the bromine is frozen or collected in a 20% sodium hydroxide solution. The reaction tube and the traps must be carried to the hood immediately after the end of the run, while the traps are still very cold, and the traps cleaned under the hood. As a safety precaution a 20% solution of sodium thiosulfate should be kept at the sink. If this solution is applied immediately to the skin after contact with bromine, no burn will result. In this respect sodium thiosulfate was found far superior to all other antidotes.

Recovery of bromine was not found feasible. Distillation of the waste bromine at room temperature by means of a current of air, with or without sulfuric acid, was not successful because the complex Br_3^- ions formed by the reaction of the bromides and bromine (*34*) so lowered the vapor pressure of bromine that it was distilled only very slowly. Distillation at 50° to 60° C. was not found desirable, as considerable water and other impurities were driven over. Because the cost of the bromine per determination was very nominal, further experiments on the recovery of the bromine were not considered worth while.

According to preliminary work in this laboratory, the application of the method to titanium or titanium alloys containing 0.5 to 1% oxygen requires that a 0.5- to 1-gram sample be brought in very intimate contact with the graphite by being crushed to a powder and mixed thoroughly with very fine graphite. The mixture is then placed in a boat containing a layer of the graphite and compressed with a flat instrument. Another layer of graphite powder is added and the mixture is again compressed. The sample is then treated with bromine in the regular manner. Titanium and titanium alloys containing more than 0.5% oxygen are very brittle and easily crushed to a powder. For the application of the method to samples containing more than 1% oxygen, a 0.5-gram sample is treated as above, but spectrographic graphite is used and after the reaction is finished the contents of the boat are ignited in air in a tared platinum crucible to eliminate the carbon. Any unreacted oxygen in the sample is then weighed as titanium dioxide.

An alternative method of calculating the blank is to run an accurate standard sample and apply the following formula:

$$B = \frac{W - 0.0275\,PM}{T}$$

where

B = blank, grams of carbon dioxide per hour
W = weight of carbon dioxide, grams
P = per cent oxygen in sample
M = weight of sample, grams
T = time for run, hours

The above formula is derived from the expression:

$$\frac{36.36\,(W - BT)}{M} = P$$

The basic reaction involved in the method as applied to titanium and titanium alloys can be represented by:

$$TiO + 2Br_2 + C \rightarrow TiBr_4 + CO$$

The reaction could also be expressed by the following equations:

$$2TiO + 2Br_2 \rightarrow TiBr_4 + TiO_2$$

$$TiO_2 + 2Br_2 + 2C \rightarrow TiBr_4 + 2CO$$

Table I. Results for Oxygen in Titanium and Titanium Alloys

Sample and Type of Alloy	Alloying Elements	Oxygen Present (Vacuum Fusion), %[a]	Oxygen Found (Proposed Method), %	Diff. between Vacuum Fusion and Proposed Method, %	Std. Dev. Proposed Method, %
WA-2, Cr-Al-Ti alloy	Cr, 5% Al, 3% C, 0.7%	0.271	0.290 0.267 0.292 0.265 0.291	+0.010	0.014
			Av. 0.281		
WA-9, RC-130B	Al, 4% Mn, 4%	0.134	0.135 0.116 0.118 0.124 0.118	−0.012	0.008
			Av. 0.122		
WA-10, comm. pure Ti		0.130	0.144 0.140 0.149 0.127 0.153	+0.013	0.010
			Av. 0.143		
WA-12, Ti-150A	Cr, 2.7% Fe, 1.3%	0.336	0.358 0.344 0.349 0.344 0.351	+0.013	0.006
			Av. 0.349		

[a] Average of results from 11 laboratories.

Evidence in favor of the mechanism indicated by the above two equations lies in the fact that any unreacted titanium oxide left in the boat is found to be white titanium dioxide rather than black titanium monoxide. If too low a temperature is used (below about 750° C.), some carbon dioxide will be formed. Titanium carbide is not formed as an intermediate in the reaction because its formation requires a temperature of 1600° to 1700° C. Bromine activates graphite and therefore increases the tendency of graphite to take up oxygen. Bromine reacts with graphite to form the absorption compound "bromine graphite" ($CBr_{0.77}$) (*34*). There is no record of carbon and bromine reacting to form carbon tetrabromide. Even if this compound were formed, it would be easily frozen out, as its freezing point is rather low (48.4° C. for the α variety and 90.1° C. for the β variety). Under the conditions of the method it is conceivable that some very volatile bromine-carbon compound is formed. The authors believe that the bromine compound removed by the zinc column is a bromine-carbon compound. This compound could not be frozen by the use of liquid nitrogen.

None of the elements that might be found in commercial titanium or titanium alloys interferes with the method. These elements include aluminum, calcium, carbon, chlorine, chromium, cobalt, copper, hydrogen, iron, magnesium, manganese, molybdenum, nickel, niobium, nitrogen, silicon, tantalum, tin, tungsten, vanadium, and zirconium. The nitrogen is probably evolved as the gas when the metal is attacked. The hydrogen is probably released as hydrogen bromide, since carbon is a catalyst for the combination of hydrogen and bromine to form hydrogen bromide. The operating temperature of 825° C. is favorable to the formation rather than decomposition of hydrogen bromide. The hydrogen bromide would either be frozen or removed by the Ascarite. Chlorine would be released as elemental chlorine or would form bromine chloride, BrCl, in the cooler parts of the reaction tube, and would either be frozen or removed by the Ascarite. The carbon remains behind as elemental carbon. The colors of the solid bromides which accumulate to some extent in the vertical portion of the reaction tube give an indication of the elements that might be present in a titanium alloy (although the effect is somewhat masked by the yellow color of the titanium tetrabromide): aluminum, white; chromium, purplish black with metallic luster; cobalt, green; copper, black; iron, brown; manganese, pink; molybdenum, reddish black; nickel, yellowish brown; niobium, purplish red; tantalum, yellow; tin, white; tungsten, violet-brown; vanadium, brownish black; zirconium, white. The colors of the solid bromides differ somewhat from the colors of the bromide vapors.

Nitrogen can be substituted for the helium, but the reaction takes longer because titanium nitride is not so readily attacked as titanium metal. Liquid air or nitrogen can be substituted for the dry ice–alcohol mixture. Chlorine cannot be used in place of bromine, because of the possible formation of carbonyl chloride by the reaction of chlorine with carbon monoxide in the cooler parts of the reaction tube (carbonyl chloride is not stable above 350° C.). Bromine and carbon monoxide do not react to form carbonyl bromide.

RESULTS

The results obtained for oxygen in four samples, "commercially pure" titanium and three representative alloys, are shown in Table I. The samples, which were in the form of $^3/_{16} \times 0.25 \times 6$ inch bars, were prepared by the Watertown Arsenal. The oxygen content of the samples was determined by the vacuum fusion method in an interlaboratory project sponsored by Watertown Arsenal in which 11 laboratories participated (Task Force on Vacuum Fusion Analysis for Oxygen, Panel on Methods of Analysis, Metallurgical Advisory Committee on Titanium). The results obtained by the proposed method check well with the results obtained by vacuum fusion.

Application of the method described to the determination of oxygen in zirconium, chromium, steels, and other materials is being investigated.

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Application of Zone Electrophoresis to Analysis of Serum Proteins

Technique for Horizontal Strip Method and Evaluation of Its Precision and Accuracy

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Successful electrophoretic separation of serum proteins requires careful standardization of technique. The present paper describes a standardized procedure, to be used with a freely suspended horizontal paper strip, which features conditioning of the apparatus and application of serum samples by means of a paper applicator strip with minimal disturbance of the established equilibrium. The precision of the method, including direct densitometric measurement of dyed proteins separated on paper strips, is equal to that of the moving boundary method. Accuracy, evaluated by analysis of mixtures of proteins, recovery of proteins added to serum, and comparison with the moving boundary method, has been found satisfactory. The good agreement between analyses of serum proteins by the paper electrophoretic and moving boundary methods seems to be the result of compensating factors.

THE simplicity of the technique and apparatus and the minute amounts of serum required make paper electrophoresis a procedure of great promise in clinical chemistry. The literature has been reviewed by Grassmann (*4*), Fisher (*2*), McDonald and coworkers (*7, 8*), Wunderly (*13*), Martin (*10*), and Block and coworkers (*1*).

The earlier studies were concerned mainly with the qualitative evaluation of changes in serum protein in disease. Efficient methods for quantitative measurement of the protein zones have further increased the value of the method. The accepted criterion for quantitative evaluation of electrophoretic patterns obtained on paper is considered by most workers to be agreement with the values obtained with the Tiselius moving boundary method. However, in comparing the results of the two methods it must be remembered that the moving boundary method has important shortcomings as an analytical tool.

There are many pitfalls also in the application of electromigration for separation of serum proteins on paper, and difficulties in the technique may explain the poor comparisons reported by some.

The present paper describes a modification of the Grassmann procedure. The results obtained by means of this method have been evaluated by analyses of mixtures of purified serum proteins, by recovery of purified proteins added to serum, and by comparison with the moving boundary method.

EQUIPMENT

The apparatus used for most of the experiments (Figure 1) is that described by Grassmann, Hannig, and Knedel (*5*) (Bender and Hobein, Munich, Germany). The buffer compartments were connected by plastic tubing to facilitate leveling of the buffer. In a second type of apparatus used (Arthur H. Thomas Co., Philadelphia, Pa.), a plastic tube connection also was installed between the buffer compartments.

The densitometer (*6*) (obtainable from Bender and Hobein or A. S. Aloe Co., St. Louis, Mo.) consists of a single-filament lamp as a light source, a photocell, a slit of about 1-mm. width, and a milliammeter with a scale calibrated to read absorbance for measurement of the output of the photocell. The paper is mounted between two thin glass plates in a metal rack for scanning. The rack has teeth which mesh with a pinion, enabling the rack to be advanced at intervals of approximately 1 mm. over the light slit. The effective length of the slit was decreased to 3 cm. to eliminate the influence of distortion of the pattern along the edges of the paper.

REAGENTS

Buffer, Ionic Strength 0.1, pH 6.8. Dissolve 29.43 grams of sodium barbital and 11.71 grams of anhydrous sodium acetate in carbon dioxide–free distilled water and add 180 ml. of 0.1*N* hydrochloric acid. Dilute to 3000 ml. Replace buffer after five runs.

Amido Black Solution. Dissolve 10 grams of the dye in 1000 ml. of a solution prepared by diluting 100 ml. of glacial acetic acid to 1000 ml. with methanol. (The dye, Amido Schwarz 10 B, was obtained from Farbenfabriken Bayer, Leverkusen, Germany. It may also be obtained from Bender and Hobein or the Arthur H. Thomas Co.)

Destaining Solution. Dilute 100 ml. of glacial acetic acid to 1000 ml. with methanol. The destaining solution can be used for

treating a series of strips, the contents of one cylinder being replaced each week.

Clarifying Solution. Mix 400 ml. of paraffin oil (heavy, water-white, Hartman Leddon Co., Philadelphia, Pa.) with 100 ml. of 1-bromonaphthalene (Matheson Co., East Rutherford, N. J.). The refractive index of this mixture should be 1.51.

Purified albumin used in these experiments was obtained from the American Red Cross in 1952. It was homogeneous when studied in a Tiselius moving boundary apparatus at pH 8.6 at several different concentrations of protein and several ionic strengths.

Gamma globulin was purchased from Sharpe and Dohme, Inc. It also was electrophoretically homogeneous.

Bromophenol Blue Solution. Dissolve 0.5 gram of the indicator in 7.45 ml. of 0.1*N* sodium hydroxide and dilute to 100 ml. with water.

PROCEDURE

The buffer compartments of the apparatus are filled with buffer with the bridge in place. The chamber is placed in the refrigerator or cold room and leveled exactly by means of a spirit level. The pinchcock on the plastic tube connecting the two inner compartments is left open to equalize the buffer levels.

For each sample to be examined, two strips of Whatman No. 1 paper, 4 × 30 cm., are numbered and marked with a pencil line across the paper, 10 cm. from one end. The paper strips are soaked in buffer, blotted, and applied to the bridge, which is removed from the apparatus for this operation. The paper is stretched just enough to keep it taut. A slight sag is unavoidable when the paper is saturated with buffer. The bridge is placed in the chamber so that the pencil lines on the papers are on the side of the cathode. The lid is placed on the chamber and a potential of 100 volts direct current is applied for at least 1 hour to condition the paper. The pinchcock on the plastic tubing is left open.

When the conditioning is completed, the serum is applied to the buffer-saturated paper by means of a narrow paper applicator strip of filter paper without removing the bridge from the apparatus (see comments on sample size). The applicator strip is taken up with forceps and dipped into the serum to be tested. The excess serum is removed by touching the inner wall of the tube as the strip is withdrawn. The applicator strip is placed gently on the top of the pencil line of the paper and allowed to remain throughout the run. It is not necessary to interrupt the current while applying the strip.

After the serum has been applied, the chamber is again covered and the connecting tube is closed by means of a pinchcock. The proteins are allowed to migrate overnight for 16 to 18 hours, at 0° to 10° C. and 100 volts. At the end of this period, the bridge with papers in place is lifted from the chamber. Excess moisture is removed immediately by blotting thoroughly with filter paper that portion of the paper which was in contact with the buffer solution. The bridge is placed immediately in a drying oven at about 100° C. for 10 minutes, with the papers remaining suspended in horizontal position.

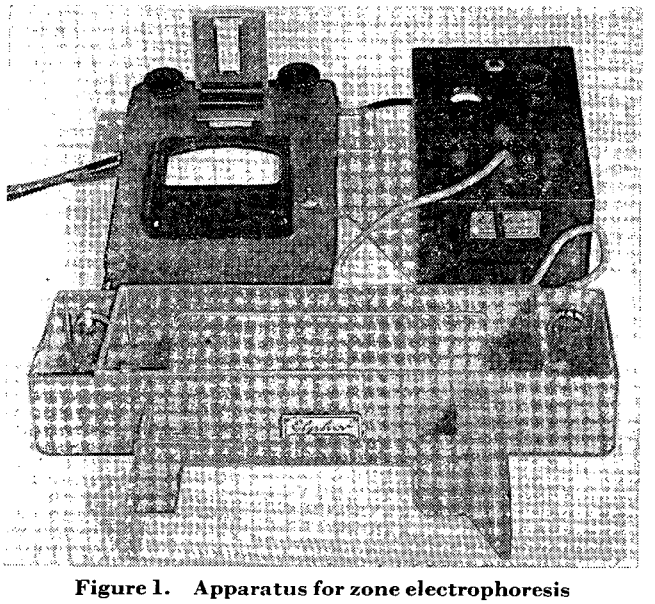

Figure 1. Apparatus for zone electrophoresis

The sections of the paper in contact with the bridge are trimmed off, and the papers are suspended by means of plastic clothespins for 15 minutes in a cylinder (9 × 30 cm.) containing Amido Black 10B solution. The paper should not adhere to the wall. A gentle air current is passed through the dye solution to keep it in constant motion. Excess dye is removed from the paper by passing the strips in succession through four cylinders containing destaining solution. The total time of destaining, which is continued until the background is nearly colorless, varies from 2 to 3 hours, depending on the freshness of the solutions. After destaining, the strips are dried at room temperature. They are trimmed further if necessary, to fit the densitometer rack, and are immersed in the clarifying solution (preferably overnight or for 2 hours in vacuo). When the strips are translucent, as judged by absence of mottling, they are inserted into the densitometer rack. The rack is moved over the light slit of the densitometer and a zero setting is made with the background color of the paper in the area preceding the albumin component. Absorbance is read at approximately 1-mm. intervals. If the pattern is to be preserved after scanning, the paper is immersed in fresh destaining solution to remove all oil.

The milliammeter readings are multiplied by 100 and plotted as a function of distance on paper ruled in millimeters. Any break in the continuity of the tracing of the gamma peak caused by the applicator strip is smoothed to give a continuous curve. Gaussian curves are completed for each of the components detected on the graph of the readings, and the surface area of each is measured by means of a planimeter. For each of the protein fractions, relative percentages may be calculated from the planimeter readings. These may be converted to absolute percentages, after determination of total protein by any standard method.

COMMENTS ON PROCEDURE

Temperature. Temperatures ranging from 0° to 10° C. were found most suitable. Although successful separations may be achieved at 20° to 25° C., the components of serum protein are more sharply defined at lower temperatures. Uniformity of temperature also is important. A failure to obtain satisfactory agreement between duplicate determinations has been traced to differences in temperature in the two sides of the chamber.

Potential Gradient. Potentials of approximately 100 volts, corresponding to a potential gradient of 4 volts per cm., gave results superior to those obtained with higher potentials for shorter periods. The latter were characterized by poor separation of albumin and alpha$_1$ globulin.

Paper. All of the studies described have been made with Whatman No. 1 paper, obtained in rolls 1.5 inches wide. The paper should be applied to the bridge with care to avoid stretching beyond the minimum required to prevent appreciable sagging. Failure to stretch the paper uniformly across its width will cause curvature of the protein zones. The conditioning of the paper improves reproducibility and may diminish trailing.

Sample Size. The size of the serum sample is important because a direct proportionality between protein concentration and densitometer reading will hold only within certain limits. Excessively high concentrations of protein in any one fraction cannot be measured quantitatively if these limits are exceeded. On the other hand, the use of too small samples will not permit accurate measurement of the smaller components.

The optimal amount of serum or urine protein for analysis according to the technique described is 400 to 700 γ. For sera with a total protein content of 6 to 8 grams per 100 ml. the applicator strip should be 2 mm. wide and about 40 mm. long. A strip of filter paper of this size, when saturated with serum, will give a pattern similar to that produced by 8 μl. of serum applied by means of a pipet. If the serum protein concentration is above or below these limits, strips of appropriate width, ranging from 1 to 5 mm., are substituted.

Method of Applying the Sample. The original technique of applying the serum from a micropipet to form a uniform streak across the paper requires skill acquired only by considerable practice, and scarcely can be done successfully unless special pipets are used. Furthermore, the use of pipets as described by Grassmann makes it necessary to blot the paper to damp dryness before applying the sample. The flow of buffer into the paper when

Table I. Precision of Electrophoresis of Serum Proteins

Technique of Serum Application and Apparatus Used	Apparatus No.	No. of Sera Studied[a]	Albumin		Globulins							
					Alpha$_1$		Alpha$_2$		Beta		Gamma	
			Std. dev.[b]	Coeff.[c]	Std. dev.[b]	Coeff.[c]	Std. dev.[b]	Coeff.[c]	Std. dev.[b]	Coeff.[c]	Std. dev.[b]	Coeff.[c]
Pipet, Grassmann	1	10	1.41	3.5	0.39	5.5	0.82	5.8	0.79	7.0	0.95	3.5
	2	10	1.57	3.4	0.65	10.9	0.61	6.2	1.21	12.3	1.26	4.5
	3	10	2.75	6.3	0.65	8.7	0.91	7.1	1.29	10.7	2.05	8.4
Strip, Grassmann	1	10	1.47	3.0	0.78	12.7	1.42	9.7	0.89	6.6	1.21	7.6
	2	10	1.60	3.5	0.89	16.7	0.59	5.1	0.82	7.3	1.83	7.0
	3	10	1.99	3.7	0.83	16.3	0.95	8.0	1.18	9.8	1.73	9.7
Strip, A. H. Thomas	1	10	2.63	5.4	0.77	20.6	1.02	10.0	0.93	8.5	1.74	6.2
Tiselius apparatus	1	6	1.90	3.1	0.75	21.0	0.80	13.8	2.58	17.6	1.38	8.4
Tiselius apparatus	2	6	1.10	2.0	0.84	15.8	1.09	10.1	0.64	5.6	0.86	4.6

[a] All determinations made in duplicate.
[b] Standard deviation of difference between pairs.
[c] Coefficient of variation.

the bridge is replaced in the chamber causes displacement of the serum band. This disturbance is avoided by use of the strip applicator technique. Perhaps the greatest advantage of the applicator strip technique is that it enables the serum to be applied to a paper that is fully saturated and equilibrated with buffer and in place in the assembled apparatus. The patterns produced when serum is applied by this technique are characterized by an even distribution of protein across the paper in uniform bands well adapted for photometric reading. Results of experiments described below suggest that accuracy may be improved by the use of this method, perhaps because of decreased trailing.

Labeling of Albumin. Labeling of the albumin by means of bromophenol blue indicator was found to be helpful. It may be done by adding approximately 1 volume of the dye to 9 volumes of serum, or by overlaying the serum applicator strip with a cotton thread (Clark No. 10) or a 0.5-mm. paper strip dipped in the dye solution. The presence of bromophenol blue does not interfere with the staining by means of the Amido Black 10B.

Trailing. An appreciable amount of serum protein remained adsorbed along the paper to form a fairly uniform trail when migration under the influence of an electric current took place at pH 8.6. Different preparations of albumin varied in the extent to which trailing occurred, depending in part on the age of the preparation. Older preparations have shown more trailing. The albumin trail will necessarily be included in measurements of the globulin components and these may be expected to show higher than their true concentrations. The effect will increase as the distance of migration increases, and as the zones spread. Thus, the optimal distance of migration is that which just suffices for adequate resolution of the globulin components. A distance of about 9 cm. has fulfilled this requirement when serum is applied 5 cm. from the center of the paper, toward the cathode.

The extent to which trailing occurs in serum is not known because the trail, if present, is obscured by the globulin components. Serum from a patient lacking gamma globulin was used to study trailing in native serum. Twenty-two of the 26 electrophoretic patterns made on paper using sera from this patient, collected at different times, showed a trail in the gamma globulin region.

Site of Application of Serum Sample. The migration of the serum albumin front during the course of a typical run is shown in Figure 2. Serum was applied on a paper strip 2 mm. wide to conditioned, buffer-saturated papers in the Grassmann apparatus in an ice chest at 9° C. Bromophenol blue was added on a second applicator strip 1 mm. wide, which was saturated with the dye and then superimposed upon the serum containing strip. A portion of the dye combined with the albumin and so marked the albumin zone. The uncombined dye migrated in advance of the albumin.

The rate of migration of the dyed albumin was rapid initially and declined somewhat as the midpoint of the paper was approached. After the midpoint was passed the rate slowed and remained constant for about 8 hours, during which the front migrated about 2 cm. The rate of migration again was accelerated as the front approached the anode. This, in part, may be due to a greater amount of buffer present in the paper close to the buffer compartments, thereby offering less resistance to the movement of the migrating substances. Tiselius and Flodin (*12*) have pointed out that polarization may lead to changes in potential gradients at the end of the paper. The bromophenol blue indicator not bound by albumin showed a similar sequence in rate of movement, although differing from the bound indicator in its more rapid migration rate.

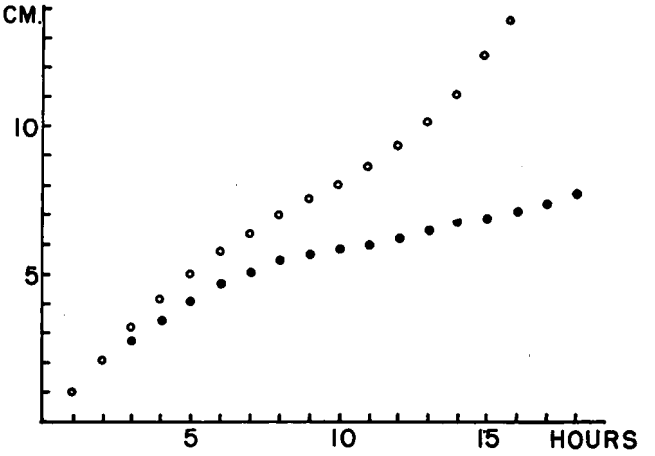

Figure 2. Migration of albumin stained with bromophenol blue

●. Albumin-bound bromophenol blue
○. Free bromophenol blue

The site of application of the sample affects the rate of separation of serum proteins (Figure 2). Although application of the sample to the midpoint of the paper is preferred if mobilities are to be estimated, the patterns obtained are characterized by poor resolution of the globulin zones and are less useful for clinical evaluation. Such patterns, of which those in the study by Mackay and coworkers (*9*) are an example, decrease the accuracy and precision with which the globulin components can be measured and conceal the presence of subcomponents of potential importance.

Removal of Excess Buffer. At the end of the migration period excess buffer must be blotted immediately from the portion of the paper that was immersed in the buffer vessels. Otherwise the flow of buffer toward the center of the paper after the bridge is lifted from the apparatus will compress the pattern.

Drying. The conditions under which the paper strips are dried are important—improper technique may cause diffuseness of the separated zones. Moreover, the combination of the protein with the dye is influenced by the duration and temperature of drying.

Satisfactory dye uptake by the protein fractions depends on the extent of heat denaturation of the proteins. Drying is started with the least possible delay after the current is turned off. None

of the protein should touch any of the metal parts of the hot oven. The paper strips should be dried in a horizontal position.

Dye Uptake. Purified human serum albumin and gamma globulin in quantities of 100 γ were applied to paper, dried for periods varying from 20 to 60 minutes at temperatures ranging from 25° to 95° C., and stained. These experiments showed that the average uptake of Amido Black 10B by albumin was 1.28 times greater than that for gamma globulin.

The length of time that the proteins are exposed to the dye solution influences the uptake of dye to an extent that differs for different proteins. Thus a pattern in contact with the stain for 2 hours showed decreased albumin and increased gamma globulin peaks. Therefore paper should be immersed in the dye solution for exactly the time specified.

PRECISION

The precision of the serum protein analyses by the techniques described has been evaluated (Table I). Three different groups, each consisting of 10 different sera, were tested in duplicate in each of three chambers using the Grassmann and Hannig apparatus, serum being applied by means of a micropipet. Four additional groups were tested, three in the Grassmann chamber and one in the A. H. Thomas apparatus, the serum being applied by means of an applicator strip. Table I also shows the results of similar experiments, made previously in this laboratory, in which six sera were studied by use of two Tiselius moving boundary apparatus of different design (*3*). In each instance the standard deviation of the differences between pairs was calculated for each of the five protein components, using the following formula:

$$\text{Std. dev.}_{\text{diff.}} = \sqrt{\frac{\Sigma(X_1 - X_2)^2}{N}}$$

where X_1 and X_2 are duplicate determinations. N equals twice the number of pairs. A coefficient of variation was calculated using the following formula:

$$\frac{100 \times \text{std. dev.}_{\text{diff.}}}{M}$$

where M represents the mean protein concentration.

The precision of albumin determinations made by zone electrophoresis in five of the seven series was as good as that of the moving boundary method. The cause of the inferior performance of two chambers was not established.

The precision of the zone electrophoresis measurements was equal to or superior to that found by the moving boundary method for all globulin fractions. This is due partly to the better resolution of the globulin components on paper and partly to the absence of the beta anomaly.

Table II. Replicates and Sample Sizes

Sample Size, μl.	Protein, γ	Albumin, %[a]	Globulins, %[a]			
			Alpha$_1$	Alpha$_2$	Beta	Gamma
8	624	55.2	5.1	9.2	11.2	19.6
5	390	55.9	4.7	10.0	9.5	19.9
3	234	56.3	4.7	7.6	12.1	19.5
1	78	53[b]	..	..	..	..

[a] All protein values expressed as per cent of total protein.
[b] Approximate.

Table III. Analysis of Mixtures of Albumin and Gamma Globulin

Experiment	Technique	Taken, γ	Albumin Recovery				Taken, γ	Gamma Globulin Recovery	
			With trail		Without trail				
			γ	%	γ	%		γ	%
A	Pipet	238	257	108	257	108	47	23	49
B	Applicator strip		229	96	212	89		42	90
C	Pipet	143	156	109	156	109	149	134	90
D	Applicator strip		146	102	119	83		144	97
E	Pipet	48	54	113	54	113	263	236	90
F	Applicator strip		63	131	29	60		238	90

Table IV. Recovery of Purified Proteins Added to Serum

Serum Total Protein, γ	Serum Gamma Globulin, γ	Gamma Globulin, γ	
		Added	Recovered
Experiment 1. Gamma Globulin Added to Agammaglobulinemic Serum			
530	0	9.4	0
470	0	19	55
480	0	56	84
660	0	101	117
Experiment 2. Gamma Globulin Added to Same Serum after Treatment			
582	48	9.4	11
525	42	19	13
563	42	60	56
525	37	90	70
Experiment 3. Gamma Globulin Added to Serum (Tuberculosis)			
300	50	47	42
480	80	105	84
300	50	147	142

	Serum Albumin, γ	Albumin, γ	
		Added	Recovered
Experiment 4. Albumin Added to Serum (Tuberculosis)			
480	194	95	83
300	121	143	126

SENSITIVITY

A single sample of serum was analyzed three times in duplicate at intervals of several days, using progressively smaller sample sizes (Table II). Albumin, alpha$_1$, and gamma globulin concentrations are in close agreement in samples of 8, 5, and 3 mμ.

A 1-μl. sample gave a correct result for albumin, but the quantities of the individual globulins were too small to be detected by the densitometer. As little as 3 γ per sq. cm. of protein, stained as described, may be measured by means of the Grassmann, Hannig, and Knedel densitometer. Smaller concentrations are not measurable, although they are still visually distinguishable.

RECOVERY STUDIES

Mixtures of Purified Proteins. Table III shows the results of the analyses of three solutions containing varying amounts of purified human albumin and gamma globulin. Experiments A and B simulate pathological conditions in which normal concentrations of albumin are combined with lowered gamma globulin. Experiments E and F simulate the conditions characterized by abnormally low albumin and very high gamma globulin concentrations. Experiments C and D represent intermediate combinations. For each experiment, 10 μl. of the protein solution were applied to the paper both by pipet and applicator strip techniques. Albumin values were calculated with and without the trail. In the experiments performed by the pipet technique no trailing was detected. However, the trailing observed in the applicator experiments may have been increased by the delay of a year in re-examining the samples which had been stored at −20° C. during the interval. The greater losses by trailing in these experiments are contrary to the usual experience with the applicator strip technique.

The gamma globulin recoveries approximated 90%, except in one experiment where the amount added was small in relation to albumin. The low recoveries of gamma globulin may be explained by a smaller uptake of dye.

Albumin recoveries were consistently high in the pipet series when trailing was negligible and in the applicator strip technique when the trail was included. When the calculations are made only by the use of the area under the albumin peak, recoveries are

Table V. Comparison of Results (Averages)

Method	No. of Sera Tested	Albumin, Grams/ 100 Ml.	Globulins, Grams/100 Ml. Alpha1	Alpha2	Beta	Gamma
		Normals				
Moving boundary	65	4.10	0.58	0.73	0.82	1.13
Paper-pipet technique	20	4.09	0.35	0.65	0.64	1.44
Paper-strip technique	24	4.14	0.30	0.61	0.72	1.23
		Patients				
		% of Total Protein				
Moving boundary	22	42.41	7.17	11.53	14.88	23.55
Paper-pipet technique	22	43.55	6.87	11.18	11.25	26.80

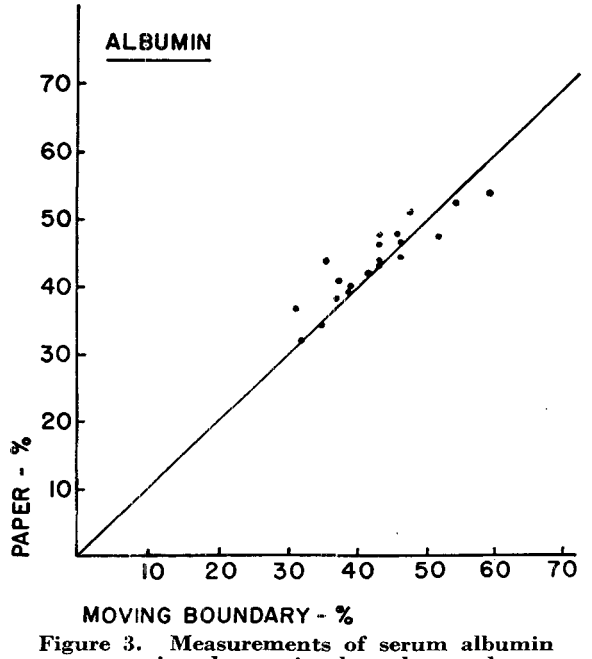

Figure 3. Measurements of serum albumin concentrations by moving boundary and zone electrophoresis methods

low. The losses by trailing are nearly constant in experiments B, D, and F despite the large differences in concentration.

These experiments indicate that the dye uptake of purified albumin and globulin in mixtures is the same as that of purified albumin and gamma globulin tested separately.

Purified Proteins Added to Serum. Gamma globulin added in increasing amounts to the serum of a patient (diagnosed as having tuberculosis) was recovered to the extent of about 90% (Table IV, experiment 3). However, when allowance was made for the lower dye uptake the recoveries are very nearly complete.

Serum lacking gamma globulin obtained from the patent suffering from agammaglobulinemia enabled recovery tests to be made without interference by the native proteins present in this zone. Experiment 1 was made when no gamma globulin was detected and the second, 2 days after the patient had received gamma globulin by vein. In the first, extra gamma globulin was recovered in three of the four experiments, probably as a result of trailing by the albumin.

The results of the second experiment were similar to those in experiment 3 in that the recoveries, although lower, approached the theoretical, particularly if corrected for the lower dye uptake of the protein added.

Recoveries of albumin added to the pathological serum approximated 87% (experiment 4). The possibility of interference with dye uptake by components of pathological sera deserves further investigation.

Comparison with Moving Boundary Method. The averaged results of moving boundary patterns of 65 healthy individuals

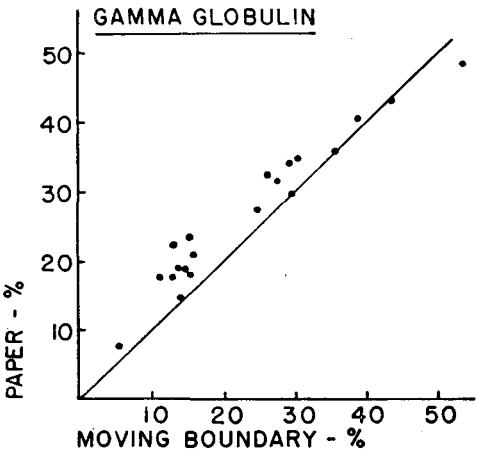

Figure 4. Measurements of serum gamma globulin concentrations by moving boundary and zone electrophoresis methods

were compared with those of 20 done by paper electrophoresis using the pipetting technique and of 24 tested on paper using the strip applicator technique (Table V). The results found by both techniques of paper electrophoresis were in agreement with those of the moving boundary method. Gamma globulin concentrations measured by the strip applicator technique are in closer agreement with those of the moving boundary method; however, this difference is not statistically significant ($t = 1.04$).

The sera of 22 patients suffering from a variety of diseases also were tested by both the moving boundary and Grassmann and Hannig methods. For these early studies, only the pipet technique was used and the paper was not conditioned. These patients include six with multiple myeloma, three with sarcoidosis, three with collagen diseases, and two with liver disease. The remainder were single examples of miscellaneous diseases. Again the results show good agreement (Figures 3 and 4). Although gamma globulin concentrations tend to be slightly higher on paper, the method accurately demonstrates changes occurring in disease.

DISCUSSION

The technique described, when used for study of serum proteins either in health or disease, gives results that approximate those obtained by the moving boundary method. This appears to be the fortunate result of compensating factors, in that losses of albumin by trailing are nearly compensated by its greater dye uptake. The same conclusions were reached by Sommerfelt (*11*). The close agreement of the gamma globulin values may be explained by lower dye binding counterbalanced by the increase of the gamma globulin area due to albumin trailing.

It is difficult to prove the existence of trailing in serum, and objection might be offered to the experiments described on the grounds that conclusions based on the behavior of mixtures of albumin and gamma globulin are not applicable to native proteins in serum. The albumin used was electrophoretically homogeneous when studied in the Tiselius apparatus and it had mobilities equal to those cited in the literature. On paper there was no immobile residue characteristic of denatured protein remaining at the point of application. Moreover, evidence of trailing was seen consistently in the gamma globulin zone of serum lacking gamma globulin. In addition, the higher values for gamma globulin concentrations, generally encountered when analyses were done on paper, support the occurrence of trailing in the presence of the full complement of serum proteins.

The numerous factors affecting the migration and separation of serum proteins on paper oblige the analyst to establish and standardize a specific technique. For such a standardization,

the results of moving boundary analyses are preferred by many; however, analyses of mixtures of albumin and gamma globulin or of sera of a group of healthy individuals also enable evaluations to be made and in some respects might be better justified. Periodic analyses of stored samples of the same serum or serum pool have proved useful.

Martin and Franglen (*10*) have described independently the use of paper ribbons for applying serum. They remove the strip after 20 minutes. Interruption of the experiment in this way disturbs the equilibrium established in the chamber and is less likely to enable transfer of all protein from the applicator strip.

Quantitative measurements of the intensity of staining can be utilized with much more confidence than mere inspection of the patterns, as some have advocated. Estimates of the amounts present in a given zone, as judged by inspection of patterns, and also as to the configuration of the peaks can be grossly erroneous. Subcomponents, however, can at times be detected better by inspection than by densitometry when the concentration of protein in such components is low.

Subcomponents in the alpha$_2$, beta, and gamma zones are frequently visible in the zones separated on paper, whereas their existence is less clearly demonstrated by the moving boundary method. This is one of many phases of serum protein behavior newly opened for study by the method of zone electrophoresis.

ACKNOWLEDGMENT

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Voltammetry at Constant Current

Application to Lead Ion in Nitric Acid Solution

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Development of the theory of constant-current electrolysis was begun many years ago, but its application to analytical chemistry has been fully recognized only recently. In order to explore the potentialities of these techniques, a versatile pen-recording instrument and a convenient electrolysis cell were designed and fabricated. In a study of the constant-current voltammetry of lead ion in nitric acid solution, an over-all precision equivalent to an average relative deviation of ±1% was obtained over the range of 0.0002 to 0.02 mole per liter. These results confirm the analytical usefulness of the technique. Although about 40% of the lead was present as the mononitrate complex in the cell solutions, no kinetic effect on the electrolysis process was observed. In general, this method, like conventional polarography, should be applicable to the analysis of electroreducible or -oxidizable materials. One possible advantage of constant-current procedures is that the electrodes can be stationary rather than dropping or rotating.

ALTHOUGH diffusion-dependent electrolysis at constant current has been studied for many years, recent papers (*1–5, 9*) have emphasized its value in the investigation of electrode processes and its analytical significance. Delahay has applied the terms "chronopotentiometry" and "voltammetry at constant current" to these measurements, in which the course of polarization of an electrode under forced constant current is followed potentiometrically as a function of time. The electrode is in contact with an unstirred solution, which contains a supporting electrolyte in addition to the depolarizer being studied. An ingenious experimental arrangement with oscillographic recording is described by Gierst and Juliard (*5*).

Delahay and coworkers (*1–4*) have made mathematical analyses of the diffusion problems corresponding to a number of specific types of electrode processes, and the resulting equations provide a sound theoretical basis for interpretation of many observed effects.

The potential-time curve recorded in the presence of a depolarizer is characterized by a transition time, during which the rate of change of potential is relatively small. This time interval is of primary analytical importance because of its dependence on concentration. In the absence of certain kinetic complications (*3*) and preceding electrochemical reactions (*1*), this relationship takes the form of the Sand equation (*10*) for zero surface concentration:

$$C = \frac{2i\tau^{1/2}}{\pi^{1/2}nF\,D^{1/2}A} \qquad (1)$$

In Equation 1, C is the bulk concentration of the reacting ion or molecule (moles per milliliter), D is its diffusion coefficient (square centimeters per second), i is current (amperes), τ is the transition time (seconds), A is the area of the polarized electrode (square centimeters), and the other symbols have their usual meanings. Thus, if i_0 represents current density, the quantity $i_0\,\tau^{1/2}/C$ is a constant characteristic of the reacting material and might be termed the "transition time constant" by analogy to the "diffusion current constant" of conventional polarography.

In the case of a reversible electrode reaction, the potential at $\tau/4$ is equal to the polarographic half-wave potential (*3*).

This paper describes a versatile pen-recording instrument for making constant-current electrolysis measurements over a range of experimental conditions. The design of a convenient electrolysis cell is described, and the utility of the technique is demonstrated by its application to the determination of lead ion in nitric acid solution.

EXPERIMENTAL

Instrument. A battery-operated constant current source and current and voltage measuring circuits were built into a standard instrument cabinet, which also housed a rapid response Brown Electronik strip chart recorder. Details of the direct current circuit are shown in Figure 1. The current and voltage measuring sections were constructed from precision resistors, those of 20 ohms and higher having ±0.1% tolerance. The electrolysis current, ranging from about 10 to 10,000 μa., is preset through a 500-ohm resistor before application to the cell. The 200-volt source was a single series of Burgess No. 5308 45-volt B batteries, mounted on Teflon and glass insulation. The shunt may be used with the panel microammeter, Weston 643, or with an external precision meter to which the proper series resistance is added.

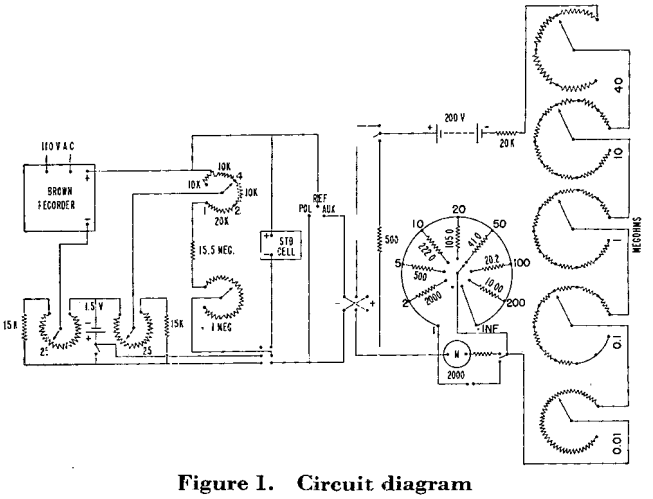

Figure 1. Circuit diagram

For recording the potential difference between the polarized and reference electrodes, full-scale sensitivities of 1, 2, and 4 volts are available with calibration made directly against the standard cell. By means of two Helipots in series with the recorder, the voltage scale may be shifted in either direction as much as the full scale value. The recorder, Model No. 153X18V-X-66N2, with 2.5-mv. range, is similar to that employed by Delahay and Mattax (*4*). The original chart drive mechanism provides chart speeds of 1 to 4 inches per second. Rapid pen response, about 1 second full scale, was retained with the high input impedances by means of circuit modifications made according to Minneapolis-Honeywell specifications (*8*).

Cell. The electrolysis cell, Figure 2, was designed to provide essentially uniform current density over the surface of the polarized electrode. The inside diameter of the lower section is 1.76 cm., and the area of mercury exposed to the solution was estimated to be 2.57 sq. cm., assuming a spherical zone determined by the uppermost point of the meniscus, observed in a cathetometer, and the circle of contact with the glass. Other features include a built-in calomel electrode which dips within 2 mm. of the mercury surface, a removable auxiliary electrode assembly with fritted-glass disk, and side arms to permit addition and withdrawal of materials while the cell is mounted in the water bath. To remove oxygen, nitrogen may be introduced through either side arm. The auxiliary anode compartment contained an agar plug prepared from 0.5*M* potassium nitrate, a pool of mercury covering the plug, and a potassium chloride solution. This anode composition was chosen to minimize contamination of the sample by diffusion of oxidation products. A cell of this design should be equally suitable for mercury pool polarography.

Materials. The lead nitrate and nitric acid were Baker analyzed reagent products. Solutions at exact rounded concentrations from 0.0001 to 0.02*M* in lead ion were prepared in approximately 0.2*M* nitric acid. *The mercury electrodes* were *triple-distilled* C.P. mercury (W. H. Curtin & Co.). Matheson prepurified nitrogen was passed over copper turnings at 450° C. and through water before entering the cell.

Procedure. The cell was mounted through rubber connections in a water bath controlled at 25.00° ± 0.01° C. The temperature control system was turned off immediately before each polarization run to prevent mechanical disturbance of the cell. After some preliminary variations in details, the following procedure was found to give reproducible results in the solutions of lead nitrate in nitric acid.

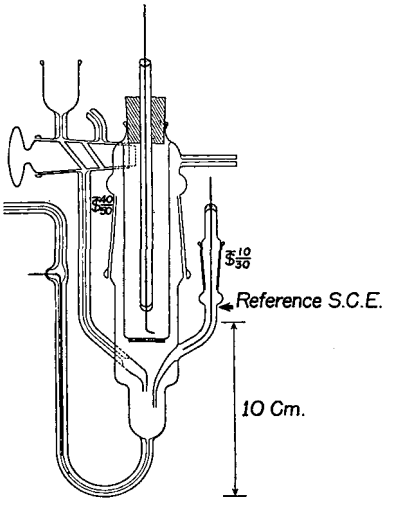

Figure 2. Electrolysis cell

For the cathode, 3.0 ml. of mercury were measured into the cell with each sample of solution. Oxygen was removed before the first run by passage of nitrogen for 15 minutes through the mercury and solution, by way of the lower capillary tube. Five minutes' bubbling, usually through the solution only, preceded each additional run. The cell was tapped, when necessary, to disperse the lead deposited at the mercury surface. The overall precision of the measurements indicates that variations in electrode area were not greater than 1 or 2%. The glass surface was not coated with a water repellant (*3*).

Figure 3 is a useful aid in selection of a suitable electrolysis current. Plots of current *vs.* transition time for several values of nC and electrode area were made from Equation 1, with $D = 1.00 \times 10^{-5}$ sq. cm. per second. With the present equipment, transition times in the range of 3 to 20 seconds are desirable. After setting the compensating voltage to place the curve in a convenient position on the chart, the preselected current is applied to the cell by the reversing switch or the adjacent battery switch. Recordings were made at a chart speed of 1 inch per second.

RESULTS AND DISCUSSION

A typical recorded potential-time curve is shown in Figure 4. Distortion by capacity effects, recorder lag, and additional electrode reactions necessitates some arbitrariness in any procedure for evaluation of transition time. The results in Table I are based on a point-tangent method introduced by Delahay and Berzins (*3*), in which τ is taken as the vertical distance from point P to the initial tangent, AB. P is selected visually by the criterion $d^2E/dt^2 = 0$. $E_{1/4}$ corresponds to a vertical distance $\tau/4$ from the curve to the tangent.

With one exception, each $\tau_{av.}$ value reported in Table I is the average of three or more recordings on a single filling of the cell, agreeing usually within 1 or 2%. Initial runs were disregarded in compiling the table because they often included some extraneous waves which disappeared in later recordings. The quantity $i\tau^{1/2}/C$ is found to be a constant, 1.44×10^3 amperes sec.$^{1/2}$ cm.^{3} per mole, with an average deviation of ±1% in the concentration range 0.0002 to 0.02*M*. Below 0.0001*M* the waves were too ill defined to measure.

Table I. Chronopotentiometric Data on Lead Nitrate in 0.2*M* Nitric Acid at 25° C.

C, Moles per Liter	i, Ma.	$\tau_{av.}$, Seconds	$E_{1/4}$, Volt *vs.* S.C.E.	$i\tau^{1/2}/C$, Amp. Sec.$^{1/2}$ × Cm.^{3} Moles^{-1} (× 10^{-3})
1×10^{-4}	0.100	3.1	−0.47	1.8
2	0.150	4.0	−0.43	1.51
5	0.250	8.4	−0.42	1.45
1×10^{-3}	0.450	10.4	−0.40	1.45
2	1.200	5.48	−0.40	1.40
5	2.50	8.33	−0.41	1.44
5	3.00	5.67	−0.39	1.43
1×10^{-2}	5.00	8.04	−0.41	1.42
1	5.00	8.26	−0.40	1.44
2	10.00	8.21	−0.40	1.43
			Av.	1.44[a]

[a] Omitting 1.8×10^3.

Existence of the ion $PbNO_3^+$ has been demonstrated recently by Hershenson, Smith, and Hume (*6*), who reported a formation constant of 3.3 at an ionic strength of 2. Delahay and Berzins (*3*) have shown theoretically that when a slow dissociation step precedes the electrochemical reaction, its influence appears as a variation of $i_o\tau^{1/2}$ with i_0 at constant concentration. An equivalent and somewhat more general function is $i_0\tau^{1/2}/C$ *vs.* i_0/C. For a reaction of the type $MX \rightleftharpoons M + X$, the per cent decrease in $i_0\tau^{1/2}/C$ from the upper to the lower limit is $100\ K_{form}\ C_x/(1 + K_{form}\ C_x)$, which is also the percentage of M present as MX in the bulk of the solution at equilibrium. K_{form} represents the formation constant of MX, and X is present in large excess. In the present case, with 0.2*M* nitrate concentration, kinetic control should cause a 40% decrease in $i_0\tau^{1/2}/C$ at high i_0C ratios. A trend of this nature was not observed in Table I, which includes i_0/C values from 175 to 389 ampere centimeters per mole. From the average $i\tau^{1/2}/C$ and the geometrically measured electrode area, a diffusion coefficient of 1.07×10^{-5} sq. cm. per second is calculated from Equation 1, assuming no kinetic control. The value based on equivalent conductance of lead ion at infinite dilution is 0.98×10^{-5}. If all of the data in Table I happened to fall in a sufficiently high i_0/C range to give only the lower, kinetically determined, limit of $i_0\tau^{1/2}/C$, then the corresponding diffusion coefficient would be 2.99×10^{-5} sq. cm. per second. This high value appears unlikely, and one concludes that if dissociation precedes the electrochemical reaction, it is too rapid to be observed easily on the equipment used.

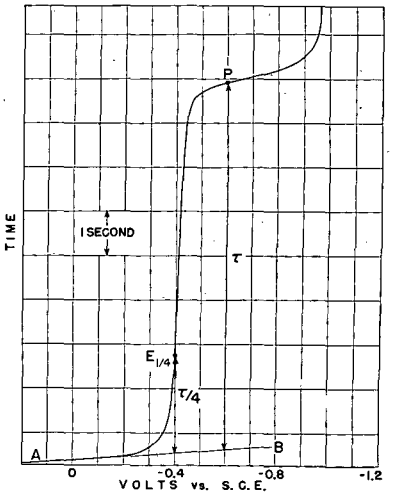

Figure 4. Recorded potential-time curve for 0.01*M* lead nitrate at 5 ma.

It is of interest to compare the quarter-wave potentials with polarographic half-wave potentials of lead (*6*): −0.386 volt *vs.* S.C.E. in 0.2*M* sodium nitrate, −0.383 volt in acidified 0.2*M* sodium perchlorate. With the accumulation of lead from repeated polarizations, a shift of the wave toward more cathodic potentials would be expected. This shift was apparent in the static potential measured before each passage of current but was negligible at the quarter wave, where $E_{1/4}$ often varied less than 0.01 volt in six recordings.

The procedure of recording repeated polarizations on the same sample has the advantage removing some surface contaminants during the first run but must be applied with caution, since traces of other interfering materials may be deposited simultaneously. It is estimated that reduction of an adsorbed monolayer of oxygen to hydrogen peroxide on the mercury pool used would require about 0.7 millicoulomb, a quantity easily measured on this instrument. In preliminary work with lead in nitric acid a peculiar overvoltage effect was observed. The first potential-time curves frequently split into two waves, one near the normal −0.4 volt, the other at −0.6 volt. In subsequent recordings on the same sample a single wave appeared in the −0.6-volt region, while the value of $i\tau^{1/2}/C$ was not affected by the change in potential. The wave returned to

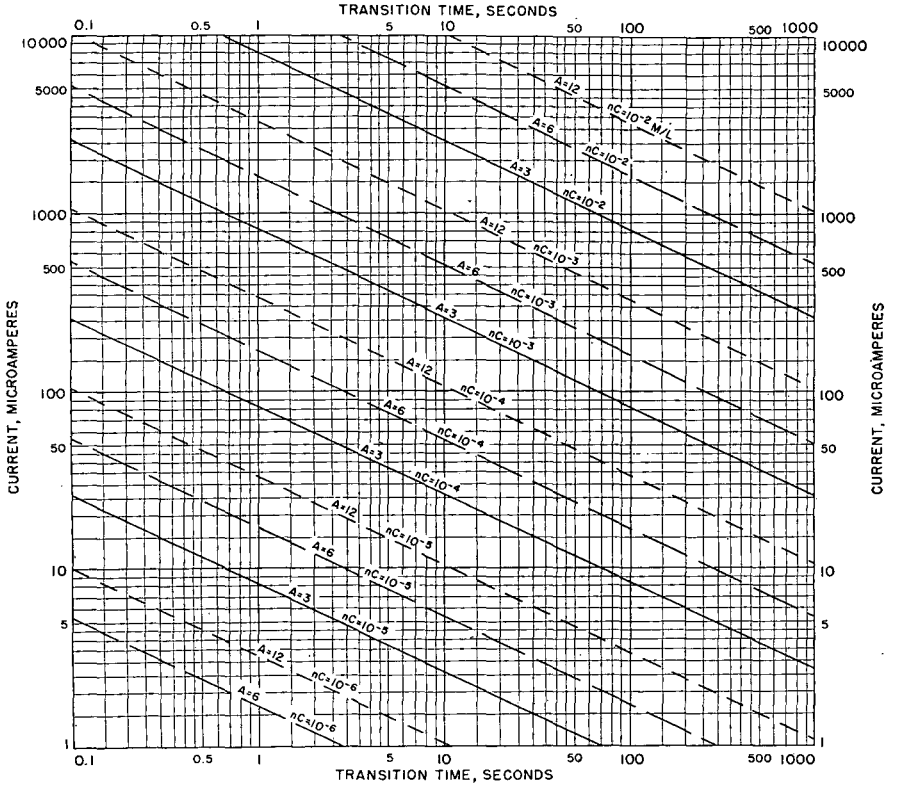

Figure 3. Transition time *vs.* electrolysis current for various concentrations and electrode areas

its normal potential range after nitrogen was passed several minutes through the mercury in contact with the solution, and the shift did not reappear. Various attempts to establish the source of this interference have been unsuccessful. It was eliminated in the measurements reported in Table I by empirical use of the bubbling procedure.

In conclusion, the results on lead ion confirm the usefulness of constant current voltammetry as an analytical method. With ordinary precautions, an over-all precision equivalent to ±1% average relative deviation in lead concentration was obtained over most of the concentration range investigated. Similar experimental technique is applicable to study of the composition and thickness of films on metal surfaces (*7, 11*).

ACKNOWLEDGMENT

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Determination of Oxygen in Metals without High Vacuum by Capillary Trap Method

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The determination of oxygen in metals by conventional vacuum fusion methods is rather slow and complicated. This paper describes a simple and rapid method which does not use high vacuum. The sample is dropped into molten platinum in a graphite crucible. Any oxygen in the sample reacts to form carbon monoxide, which is swept out by a stream of argon at atmospheric pressure. A modified form of Schütze's reagent oxidizes the carbon monoxide to carbon dioxide, which is condensed in a capillary trap and measured with a capillary manometer. The apparatus is sensitive to 0.3 γ of oxygen. Cuprous oxide samples gave 99% of the theoretical value. Results are given for samples of iron, steel, aluminum, and thorium. A determination usually takes 12 minutes.

OXYGEN in metals is usually determined by so-called vacuum fusion methods (*6*). Essentially, the sample is fused in vacuum in an induction-heated graphite crucible, often with a flux of molten metal, and the gaseous products are collected by a fast diffusion pump and analyzed, oxygen from the sample appearing chiefly as carbon monoxide. A McLeod gage is used for the final measurement. The outstanding advantage of the method is high sensitivity, fractions of a microgram being easily measured with a McLeod gage. Objections to the method, which have limited its use somewhat, are the complicated apparatus, the necessity for a specially trained operator, and the tedious procedure. It is difficult to adapt the method to routine work; each sample commonly requires an hour or more, and much time is spent in such preliminary work as changing crucibles, loading samples, and reducing the blank to a constant value.

A method not involving vacuum was used by Singer (*4*), who swept a stream of nitrogen over the crucible, and after oxidizing carbon monoxide to carbon dioxide, determined it gravimetrically, as in combustion methods for carbon. Because of its relatively poor sensitivity, this method is limited to large samples.

The present paper describes a method which eliminates high vacuum and most of its associated troubles, yet is sensitive to less than 1 γ of oxygen. The sample is dropped into a bath of molten platinum in a graphite crucible. A stream of argon at atmospheric pressure removes the carbon monoxide and carries it through a modified form (*5*) of Schütze's reagent (*3*), which converts it to carbon dioxide. The carbon dioxide is condensed out in a capillary trap and measured with a capillary manometer. The desired sensitivity is achieved by using a small volume rather than by measuring low pressures. Only a mechanical pump is used, and only the capillary trap is evacuated.

A 10-minute period is sufficient for substantially complete recovery of oxygen from many samples, and the whole determination takes only 12 minutes. The crucible can be changed quickly, and the blank is restored to its operating level in an hour or two. Samples are introduced one at a time, through a

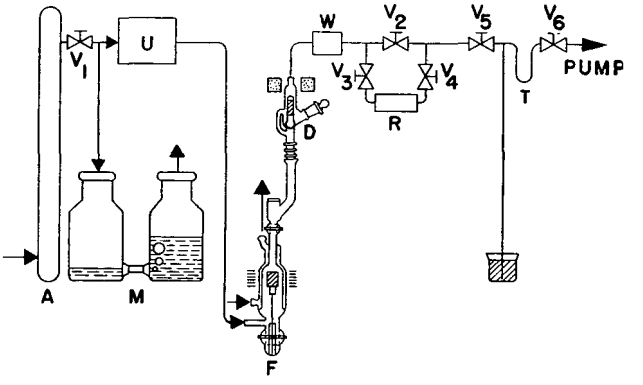

Figure 1. Schematic diagram of apparatus

A. Ascarite and magnesium perchlorate
D. Sample holder
F. Induction furnace
M. Manostat
R. Modified Schütze reagent
T. Capillary trap and manometer
U. Uranium furnace
V_1, V_2, V_3, V_4, V_5, V_6. Brass bellows type valves
W. Glass wool and magnesium perchlorate

magnetic sample holder. Because of the use of atmospheric pressure, the apparatus is simple and rugged; parts can be replaced without glass-blowing.

The unusual choice of platinum as a flux was based on its low volatility. Most samples require a flux, either to prevent evaporation of the sample or to avoid formation of a carbide "cinder" which keeps the oxide from reacting (*8*). Iron and tin, often used, evaporate to a troublesome extent, especially at higher temperatures. Among metals having the lowest vapor pressures (*2*), platinum is the only one that can be melted in a graphite crucible below 1800° C., without forming a solid carbide. The only objection to platinum is its cost; however, it can be recovered.

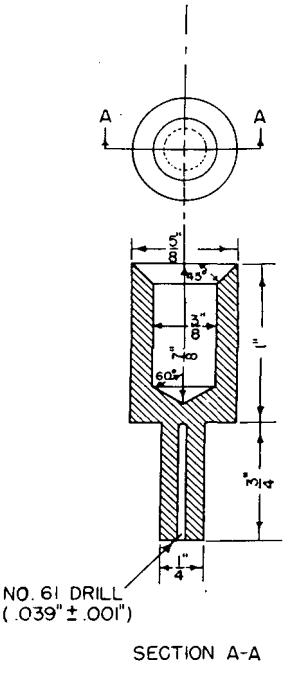

Figure 2. Graphite crucible

Because the fundamental reaction in the capillary trap method is the same as in vacuum fusion, it should have the same applicability. This paper gives examples of its application to iron, steel, aluminum, and thorium. The accuracy was tested by analysis of a pure compound, cuprous oxide, which gave 99% of the theoretical oxygen value.

The only disadvantage of the capillary trap method, as compared with vacuum fusion, is that it cannot readily be modified to permit the determination of hydrogen and nitrogen. Hydrogen is not affected by the reagent.

APPARATUS

Figure 1 is a schematic diagram of the apparatus.

The parts are connected by 0.25-inch copper tubing; glass-to-metal connections are made by short pieces of neoprene tubing. Brass bellows-type needle valves, used instead of stopcocks, permit better flow control and on the whole are more reliable, but they occasionally develop leaks, and therefore are mounted so as to be easily replaced. All glass parts can be replaced without glass blowing, thus minimizing shutdown time.

The entire apparatus up to valve V_5 is filled with argon at slightly more than atmospheric pressure. The manostat, *M*, made from two 4-liter aspirator bottles and filled with silicone oil, maintains this pressure when the apparatus is not in use; its large volume compensates for barometric changes or slow leaks. During operation, valve V_1 is adjusted so that argon bubbles out slowly through *M*. Welding grade argon is purified by passing through the tube, *A*, containing Ascarite and magnesium perchlorate (probably unnecessary), and through hot uranium in the furnace, *U*. This consists of a 1-inch nickel tube, packed with uranium turnings, and heated over 12 inches of its length by a Nichrome winding. The temperature at the center is 625° C.; higher temperatures give no improvement in the blank.

From the hot uranium treatment, the argon passes upward through the induction furnace, *F*, and sample holder, *D*. This direction of flow is essential, to prevent any oxygen that enters the sample holder from reaching the crucible. The tube carrying argon to the induction furnace is of glass rather than metal, because it passes close to the heating coil.

The induction furnace, *F*, is made of clear silica and is water-jacketed, including the constricted part at the top which guides samples into the crucible. This avoids outgassing or "getter" effects sometimes encountered when the sample dropping tube is heated by the crucible. Figure 3 shows details of the furnace.

The dimensions of the graphite crucible are shown in Figure 2. It is mounted on a 0.040-inch tungsten rod, supported by a capillary sealed into the base of the furnace, which is a spherical joint, lubricated with silicone grease, and large enough to pass the crucible. The crucible can thus be replaced without dismounting the furnace. The crucible contains about 6 grams of platinum Scrap platinum can be used, but it should be free from base metals.

The crucible is heated by a Thermonic 10-kw. oscillator, with the addition of a gang of Variacs to control the plate voltage. The heating coil consists of ten turns of flattened copper tubing. Normally, the crucible is heated just above the melting point of platinum, or about 1800° C. An optical pyrometer may be used to check the temperature, but accurate measurements are questionable because of darkening of the window. A more accurate procedure is to observe the melting of the platinum, using a dark glass.

The sample holder, *D*, is connected to the furnace by an adaptor having a $ 19/38 joint at the top and a spherical 18/9 joint at the bottom. Both parts are of borosilicate glass. The adaptor has a plane window through which the crucible may be observed. The side arm of the sample holder, through which samples are inserted, has a $ 19/38 stopper, which is not greased. Under the small pressure difference existing, leakage is negligible. The sample is dropped by lifting the iron-cored plunger by means of a solenoid, which is controlled by a rheostat for smooth operation. A bypass tube is provided, so that the plunger can be left seated when not in use. Details are shown in Figure 3.

From the sample holder the argon stream passes through a tube, *W*, containing glass wool, to filter out soot from the crucible, and magnesium perchlorate, to remove traces of moisture which otherwise appear in the blank.

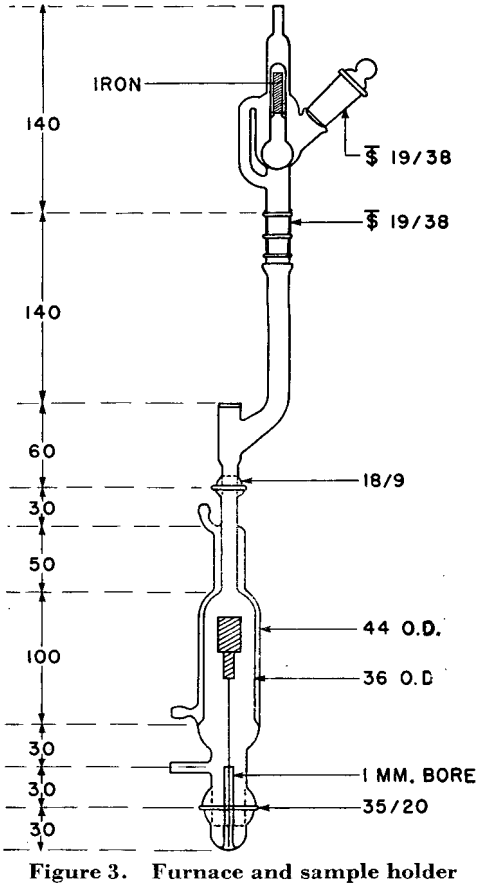

Figure 3. Furnace and sample holder

Furnace silica, other parts borosilicate glass. Dimensions in millimeters

Next, the argon flows through the oxidizing reagent, *R*. Valves V_2, V_3, and V_4 permit the reagent to be bypassed when desired, as when a new crucible is broken in. Modified Schütze reagent is prepared (*5*) by saturating silica gel with an aqueous solution of iodic acid, drying, moistening with sulfuric acid, and heating at 220° C. in a slow stream of dry air at reduced pressure (1 mm. or less), until a golden color is produced.

The reagent must be protected from the air, as it is very sensitive to moisture. It oxidizes carbon monoxide to carbon dioxide on contact, at room temperature. The slow exhaustion of the reagent can be followed by the spread of the brown iodine stain. The modified reagent differs from the original (*3*) in having a coarse form, minimizing resistance to gas flow.

The capillary trap, T, is sealed into valves V_5 and V_6 with Apiezon W wax. It is important that the valves be oriented so that the side with small volume is next to the trap. The dimensions of the trap and manometer are shown in Figure 4; however, the length of the manometer depends on the barometric pressure, and in general will be longer than at the high altitude of Los Alamos. The length below the zero mark should be 1 or 2 cm. more than the highest barometric pressure expected. The lower end of the manometer is immersed in a beaker of mercury, and the level is adjusted, by adding or removing mercury with a medicine dropper, so that the meniscus is on the zero mark when the system is evacuated (V_5 closed, V_6 open). A millimeter scale is placed against the manometer, reading downward from the zero mark.

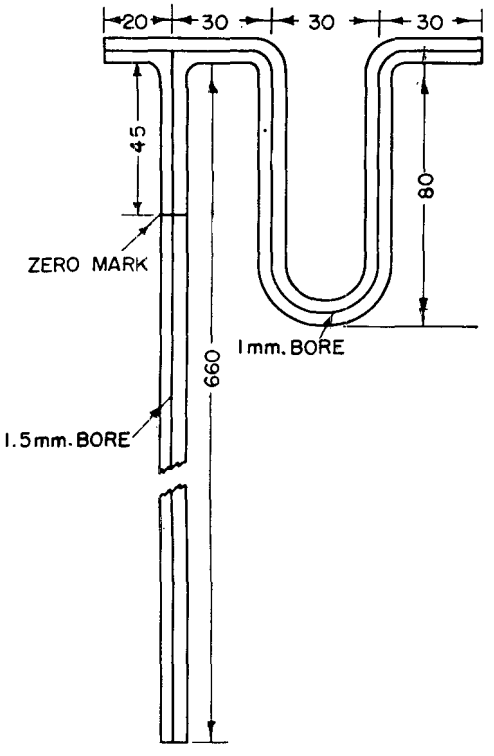

Figure 4. Capillary trap and manometer

Borosilicate glass. Dimensions in millimeters

The manometer and trap are so arranged that the combination acts as a flowmeter. A mercury depression of 50 mm. corresponds to the standard flow rate of 1 liter in 10 minutes.

From valve V_6, a copper tube leads to the mechanical vacuum pump. Almost any type of pump can be used, as a vacuum of 0.1 mm. is adequate.

CALIBRATION

If the volume of the trap above the zero mark is V, the cross section of the manometer bore is S, and the manometer reading is x, the weight of oxygen is given by

$$W = 16x(V + Sx)/RT \quad (1)$$

the formula weight of 16 being used because only one atom of oxygen per molecule of carbon dioxide comes from the sample. Expressing weight in micrograms and linear dimensions in millimeters, and taking the temperature as 25° C., this becomes:

$$W = 0.00086\,(Vx + Sx^2) \quad (2)$$

The cross section, S, if not known, may be determined from the weight of mercury occupying a measured length in the capillary. Because of the metal valves, the volume of the trap, V, cannot be measured in this way. It is determined from S by Boyle's law, as follows:

With a rubber tube, connect a leveling bulb filled with mercury to the bottom of the manometer. Arrange for one of the two manometer valves to open to the atmosphere—for example, by removing the stopper of the sample holder and opening valve V_2. With the trap open to the atmosphere, raise the mercury to the zero mark. Read the atmospheric pressure, h, on a near-by barometer. Close the valve to seal the trap from the atmosphere, and lower the leveling bulb to near the bottom of the manometer. Note the manometer reading, x_1, open the valve to the atmosphere, and read the new level, x_2. The initial pressure in the system was h, the pressure after expansion was $h - x_2 + x_1$; hence by Boyle's law,

$$Vh = (V + Sx_1)(h - x_2 + x_1) \quad (3)$$

Solving for V,

$$V = Sx_1\,(h - x_2 + x_1)/(x_2 - x_1) \quad (4)$$

Four determinations of V usually agree within a range of 1%.

Values of W can now be calculated from Equation 2. It is convenient to calculate a table of W as a function of x. With the given dimensions, V is about 740 cu. mm., and S is about 1.7 sq. mm. The useful length of the scale is 510 mm., giving a capacity of about 700 γ. The sensitivity, reading the scale to the nearest 0.5 mm., is 0.3 γ near the zero and 1.2 γ at full scale. The calibration is probably accurate within 1% (a 3° temperature change produces an error of 1%).

PROCEDURE

Flushing. Whenever the apparatus has been idle for more than a few hours, it must be flushed to reduce the blank.

Turn on the argon and adjust valve V_1 so that argon bubbles out slowly through the manostat. Start the vacuum pump, open valve V_6, and adjust the mercury column to the zero mark by adding or removing mercury, tapping to free the meniscus. Open V_2, leaving V_3 and V_4 closed, and adjust V_5 to give a manometer reading of 50 mm., corresponding to a flow rate of 100 ml. per minute. Be sure argon still bubbles through M; adjust V_1 if necessary. Turn on the cooling water to the furnace, and begin heating the crucible at 1800° C. After 20 minutes, open V_3 and V_4 and close V_2, to pass the gas stream through the reagent. Continue flushing for 10 minutes, then make a trial blank run.

Flush a new crucible with the reagent bypassed, for half an hour below the melting point of platinum, starting at a red heat and increasing gradually. After melting the platinum, continue flushing for an hour, then proceed as above.

Blank. With argon flowing at the standard rate, place a Dewar flask of liquid nitrogen over the trap, and set a timer for 10 minutes. After 10 minutes, close V_5, wait a few seconds, and close V_6. Remove the liquid nitrogen, warm the trap with warm water, and dry it. Tap the manometer and read to the nearest 0.5 mm. Open V_6, and check the zero setting, if it seems necessary.

Except on days of rapid barometric change, it is usually not desirable to change the initial zero setting. Any gradual zero shift will affect the blanks and the samples alike, and so cancel out, to a good approximation. The zero should not be adjusted while the trap is chilled, as the mercury then tends to pause below the zero mark, reaching its true level only slowly, no doubt because of some capillary effect.

Blank determinations should be repeated until successive blanks agree within 0.5 mm. The value of the blank varies in the range of 2 to 7 γ, depending on the history of the furnace. It decreases when the apparatus is in constant use, and increases when it stands idle. Changing crucibles does not greatly affect the blank, but changing or cleaning the furnace may increase it considerably.

Sample. With V_5 closed and V_6 open, turn off the induction heater (never approach the furnace with the high voltage on), remove the stopper of the sample holder, drop in the sample, and replace the stopper with a slight twist to seat it firmly. Turn on the heater, adjust V_5 to give a reading of 50 mm., chill the trap, and set the timer for 10 minutes. Operate the solenoid to drop the sample. After 10 minutes, close V_5, then V_6, warm the trap, and read the manometer as described above.

Some light or thin samples tend to miss the hot crucible because of the strong convection currents around it. This may be avoided by dropping the sample just before turning on the heat. This variation in the procedure has not been found to alter the results. Dropping the sample into the hot crucible has the advantage that the fall and reaction of the sample may be observed, using a dark glass.

The loading of the sample and the temporary cooling of the crucible do not affect the value of the blank; therefore it is unnecessary to repeat these operations in a blank run. In working with a new type of sample, blank runs should be made after every sample, until its behavior is known. If it is established that the reaction is substantially complete in 10 minutes (no significant increase in the blank), then samples may be analyzed in succession, with only an occasional blank determination. For some samples, it might be desirable to increase the reaction period; the blank would then be correspondingly larger.

With a 10-minute reaction period, the total time for a determination is about 12 minutes.

Calculations. The manometer readings for blanks and samples are converted into micrograms of oxygen, W, by Equation 2, or by a calibration table. The W value for each sample is corrected by subtracting the W corresponding to the blank. If the blank shows a uniform trend, the correction for each sample is obtained by interpolation between the last blank and the next. If there is no trend, an average blank is used. The oxygen content is given by:

$$\% \text{ oxygen} = \frac{1}{10} \frac{W \text{ (sample)} - W \text{ (blank)}}{\text{weight of sample (mg.)}} \qquad (5)$$

Table I. Oxygen Values on Cuprous Oxide

(1400° C., no flux)

Sample Weight, Mg.	Oxygen, %
1.35	11.00
1.69	14.64[a]
1.42	11.06
0.79	11.09
1.91	11.08
95% confidence interva	11.07 ± 0.065
Theory, Cu_2O	11.18
Error	− 0.11

[a] Rejected; probably contaminated.

RESULTS AND DISCUSSION

In presenting the data, the standard deviations and confidence intervals given were estimated from the median and range (*1*). All rejected values exceeded the rejection quotient for the 90% confidence level (*1*).

The accuracy of the method was checked by analyzing a known compound, cuprous oxide, prepared by igniting copper oxide wire at 1000° C. in air. Its purity was indicated by its transparency under the microscope. Small cuprous oxide samples were analyzed in the empty crucible (without a flux) at 1400° C. The results appear in Table I. Both the accuracy and the precision are very good in comparison to vacuum fusion methods, the recovery of oxygen being 99.0% of the theoretical. Of the oxygen recovered, about 99% was obtained in the first 10-minute period, but because of the large amount of oxygen involved, a second and sometimes a third run were needed to restore the blank to its original value.

Table II gives the results of analyses on iron and steel, using the platinum flux. The sample designated "NBS 6" is a section of a 1-inch steel rod from the cooperative oxygen determination program of the National Bureau of Standards (*7*). Because of radial segregation of oxygen within the rod, it was recommended (*7*) that an entire section be used as a sample. This was not feasible with the present apparatus, designed for small samples.

Table II. Oxygen Values on Iron and Steel

(1800° C., platinum flux)

Sample	Sample Weight, Mg.	Oxygen, %
NBS 6	40– 80	0.008 0.009 0.011
	160–240	0.012 0.008 0.011
	95% confidence interval	0.010 ± 0.0015
	Standard deviation	0.0015
	NBS best value	0.007
	Range of cooperative values	0.003 − 0.0085
Cenco	80–100	0.160 0.160 0.164 0.155 0.157 0.158 0.176[a] 0.158
	95% confidence interval	0.158 ± 0.0026
	Standard deviation	0.0032

[a] Rejected.

Table III. Oxygen Values on Aluminum and Thorium

(1800° C., platinum flux)

Sample	Weight, Mg.	Oxygen, %
Al	275.2	0.0102
	238.7	0.0098
Th	91.0	0.124
	87.2	0.120

The disk was therefore sampled by sawing out a roughly radial slice and cutting the slice into small pieces, which should give a fairly representative sampling. The pieces were etched with hydrochloric acid and rinsed with water and acetone, and appeared bright and clean at the time of analysis. Each sample was one piece.

Making due allowance for the method of sampling, the results on NBS 6 are somewhat higher (certainly not lower) than those obtained in the cooperative program. This would indicate that there are no serious losses such as are sometimes caused, for example, by manganese (*6*, *7*) (NBS 6 contains 0.47% manganese). Two ranges of sample size were used, with no significant effect on the results.

The sample designated as Cenco was "iron wire for standardizing" sold by the Central Scientific Co. It was cleaned in the same manner as the steel samples. Considering its high oxygen content, the results are fairly uniform, the standard deviation being 2% of the total oxygen. The oxygen content of this particular sample is high enough to affect its accuracy as a volumetric standard.

With both the iron and steel samples, a single 10-minute reaction period was sufficient for complete recovery of the oxygen. This permits an operating schedule of four determinations and one blank per hour.

Table III gives the results of service analyses on pure aluminum and thorium. In both cases the recovery of oxygen was about 99% complete in 10 minutes.

Experiments with titanium were less successful. Pure crystalline titanium (iodide process) was used. The first sample reacted with a brilliant flash, giving a reasonable result (0.0055%) and no increase in the blank. Succeeding samples, however, gave progressively lower results, with an increase in the blank, while dark spots appeared on the surface of the platinum. This rapid exhaustion of the flux is probably due to the low density of the titanium carbide, which floats on top of the platinum. To overcome this effect, it would be necessary to add more platinum with each sample. Walter (*8*), using tin as a flux in the vacuum-fusion determination of oxygen in titanium, found it necessary to add tin with each sample.

An attempt was made to use tin as a flux in place of platinum, for the analysis of titanium. The crucible contained 4 grams of

tin at about 1750° C. At this temperature, the tin evaporated rapidly, and a stream of condensed tin particles was visible, depositing throughout the apparatus. A titanium sample reacted with a small flash and considerable turbulence, but no oxygen was obtained above the blank level. This surprising result may be due to a getter action of the tin vapor, which does not occur in the vacuum fusion method because the vapor does not reach a high enough concentration in the crucible. If this explanation is correct, it is an added reason for using a noble metal as a flux.

CONCLUSION

The capillary trap method should be an acceptable substitute for vacuum fusion in the determination of oxygen in many metals, with good sensitivity and precision, and great improvement in speed and simplicity.

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Differential Thermal Analysis of Inorganic Compounds

Nitrates and Perchlorates of the Alkali and Alkaline Earth Groups and Their Subgroups

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Differential thermal analysis has been used in investigating the thermal decomposition of inorganic oxidants to determine and characterize their behavior and reactions at elevated temperatures over the range of ambient to approximately 800° C. Although the curves obtained by this technique are unique and characteristic for the individual compounds, relatively little work has been reported on the use of differential thermal analysis for this purpose other than in the field of mineralogy. The results of a study of the nitrates and perchlorates of the alkali and alkaline earth groups and their subgroups are described. The parameters established for the system were maintained constant and a heating rate of approximately 15° C. per minute was employed to permit comparison of the curves under uniform conditions. The thermal reactions reported include dehydration, crystalline transition, fusion, bubbling and/or boiling, and the various stages of visible decomposition. The transition temperatures for salts which undergo crystalline transformations have been determined from the differential thermal analysis curves and are compared with reported values.

ALTHOUGH the techniques of differential thermal analysis have been widely exploited in the study of clays, minerals, and soils, in relatively little work has this method been utilized to investigate and characterize the thermal decomposition of inorganic compounds (*6–8, 12*). Differential thermal analysis involves measuring the temperature difference between an inert reference compound—e.g., ignited alumina—and the material under study as they are both heated to elevated temperatures at a constant rate. Since a reference material is selected which will undergo no thermal reactions over the temperature range under investigation, any endo- or exothermal changes of the sample will cause its temperature to be lower or higher, respectively, than that of the reference material, resulting in endo- or exothermal differential temperatures which are recorded as a function of the sample or furnace temperature.

Techniques such as absorption and emission spectroscopy, or electron and x-ray diffraction can be used for the solid state identification of inorganic substances, but they are inapplicable to thermal reactions involving endo- or exothermal processes characteristic of the thermal decomposition of inorganic substances. These methods are restricted to the identification of reactants, intermediates, and end products, whereas by differential thermal analysis a continuous record is obtained, over the whole temperature range under consideration, of the thermal effects accompanying melting, boiling, crystalline transition, dehydration, decomposition, oxidation, and reduction. Differ-

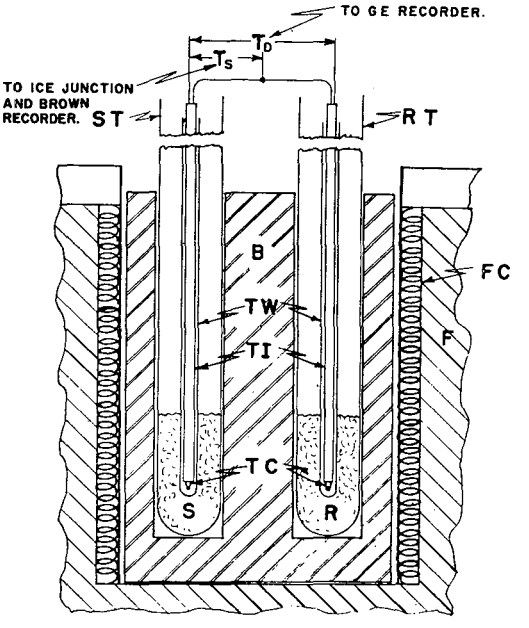

Figure 1. Differential thermal analysis furnace, block, and sample tube arrangement

F. Furnace, 110 volts, 550 watts
FC. Furnace coil and core
B. Block, steel, 2⅞ × 4 inches
ST. Sample tube, borosilicate glass, 18 × 150 mm.
RT. Reference tube
TW. Thermocouple well, borosilicate glass
TI. Thermocouple insulating tube, round, double bore
TC. Thermocouple, Chromel-Alumel, B&S No. 28
T_D. Differential temperature
T_S. Sample temperature

ential thermal analysis is therefore worthy of consideration as an adjunct to the multitude of techniques available for characterizing substances, in this case by their relative thermal stabilities and unique behavior at elevated temperatures.

A large variety of inorganic oxidants is used in the formulation of pyrotechnic compositions, which are essentially intimate physical mixtures of these oxidants with finely divided fuels—e.g., metal powders. When ignited, these compositions burn to produce large amounts of light, heat, smoke, and sound. In order to investigate pyrotechnic chemical reactions which take place at high temperatures and involve rapid heating rates, it is important to study the thermal decomposition of the many oxidizing agents employed. These oxidants include the alkali and alkaline earth metal nitrates, perchlorates, chlorates, chromates, oxides, peroxides, and oxalates, as well as analogous compounds of ammonia, some heavy metals, and transition elements. A preliminary characterization of these oxidants by their thermal instability at elevated temperatures by differential thermal analysis proved satisfactory, in that the curves obtained could be interpreted on a basis of the transition, fusion, and decomposition phenomena of the respective compounds.

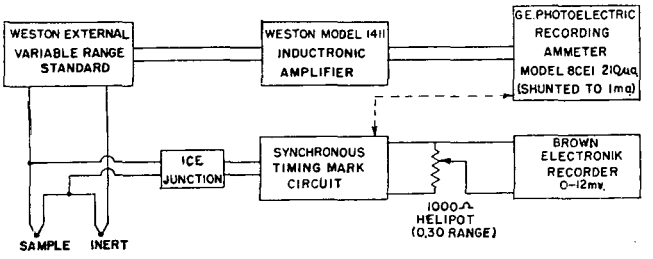

Figure 2. Differential thermal analysis instrumentation

A comprehensive differential thermal analysis of many of the afore-mentioned inorganic compounds has been undertaken, as a review of the literature showed a dearth of this type of physical data (*1, 2, 10*). The many discrepancies found in handbooks and other literature pertinent to decomposition temperatures can be attributed to the diversity of thermal parameters used by the various investigators, which in many cases are incompletely described. The latter difficulty has been overcome in this investigation, as the compounds have been studied under uniform conditions, with particular emphasis on maintaining a constant heating rate. The major objectives were to determine the reproducibility of the curves obtained by this method, to observe the phase changes and stages of decomposition undergone by the compounds at predetermined heating rates, and to characterize the various types of decomposition phenomena. In this paper are presented the differential thermal analysis curves for the thermal decomposition of the nitrates and perchlorates of the alkali metals, alkaline earths, and their subgroups.

EXPERIMENTAL

The chemical reagents used were purchased as C.P. or reagent grade, with the exception of rubidium and cesium perchlorates, which were prepared in the laboratory from the corresponding nitrates. Sources and grades of the chemicals are as follows: rubidium nitrate, C.P. (A. D. MacKay); silver nitrate and ammonium perchlorate, C.P., and strontium nitrate, tested purity, (Eimer and Amend): cesium nitrate, C.P. (Foote Mineral Co.); beryllium nitrate, sodium and magnesium perchlorates, C.P. (Fisher Scientific Co.); lithium, copper(II), silver, calcium, strontium, zinc, and mercury(II) perchlorates, reagent (G. F. Smith Chemical Co.); ammonium nitrate, potassium and barium perchlorates, analytical reagent grade (J. T. Baker Chemical Co.); lithium, sodium, potassium, magnesium, calcium, barium, zinc, cadmium, mercury(I), and mercury(II) nitrates, analytical reagent grade (Mallinckrodt Chemical Works); copper(II) nitrate, reagent (Merck & Co.). Those materials which were obtained as large crystals were pulverized to approximately 100 mesh with a mortar and pestle. All samples not denoted as hydrates were then dried for at least 2 hours at 110° C. The aluminum oxide used as an inert reference compound was heated at approximately 650° C. for 1 hour.

INSTRUMENTATION AND PROCEDURE

The furnace, block, and tube arrangement are illustrated in Figure 1. An amount of sample, *S*, equivalent to a depth of 3/4 inch (about 5 grams) is introduced into a borosilicate glass test tube, *ST*, and an equal amount of ignited alumina, *R*, into a similar tube, *RT*. The tubes are placed in two adjacent holes ($^1/_8 \times 3$ inches) drilled into a cylindrical tool steel block, *B* ($2^1/_8 \times 4$ inches) set in the core well, *FC*, of a resistance furnace, *F* (Hevi-Duty Electric Co., Type 84, 110-volt, 550-watt). Identical Chromel-Alumel thermocouples, *TC*, with porcelain double-bore insulators, *TI*, are contained in borosilicate glass thermowells, *TW*, inserted into the center of the test tube contents. The thermocouples are series-connected and used to measure the differential temperature, T_D, between the sample and the reference alumina.

A schematic diagram of this instrumentation is shown in Figure 2. After amplification by a Weston Inductronic amplifier (Model 1411, Type 1), the differential temperature is recorded on a 210-μa. photoelectric recording microammeter (General Electric Co., Model 8CE1, shunted to 1 ma. by a 15-ohm resistor) as a function of time. The sensitivity of the recorder to temperature differentials is varied by an external range standard on the Weston amplifier. The minimum range employed was ±20° C. The thermocouple inserted in the sample under investigation is also used to measure the sample temperature, T_S *vs.* an ice junction reference point and is connected to a Brown Electronik recording and controlling strip chart potentiometer (12-mv. span) through a 1000-ohm (10-turn, 0.5%) Helipot. The latter is used as a voltage divider to obtain the equivalent temperature span required. A dial setting of 0.30 on the Helipot provides a full scale span of 0° to 950° C. with the Chromel-Alumel thermocouple.

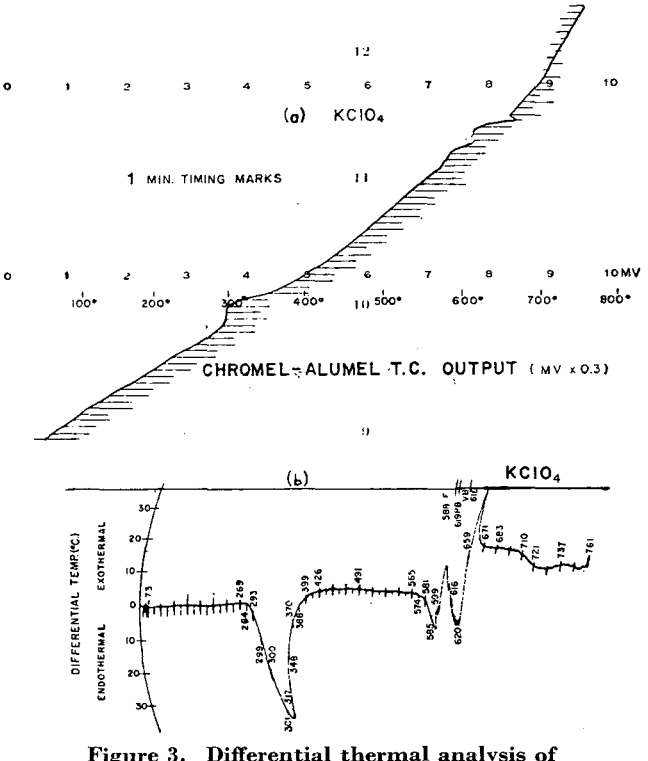

Figure 3. Differential thermal analysis of potassium perchlorate

a. Sample temperature curve
b. Differential temperature curve

The Chromel-Alumel thermocouples, prepared from large spools of wire, were periodically calibrated with the Brown recorder against boiling water and National Bureau of Standards melting point standards, over a temperature range of ambient to 660° C. The accuracy and reproducibility were found to be approximately ±1° C.

The sample temperature and differential temperature curves

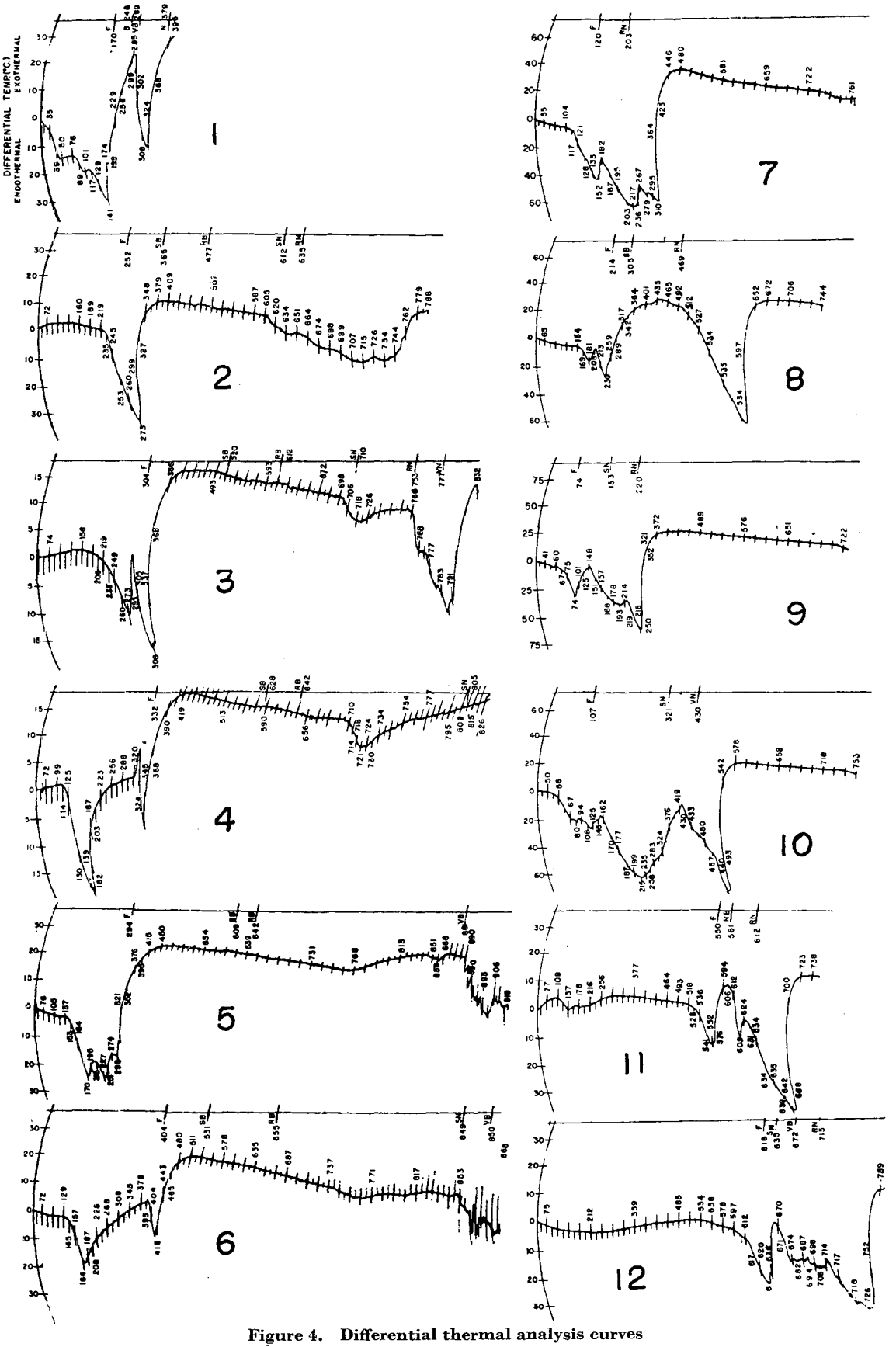

Figure 4. Differential thermal analysis curves

1. NH_4NO_3
2. $LiNO_3$
3. $NaNO_3$
4. KNO_3
5. $RbNO_3$
6. $CsNO_3$
7. $Cu(NO_3)_2 \cdot 3H_2O$
8. $AgNO_3$
9. $Be(NO_3)_2 \cdot 3H_2O$
10. $Mg(NO_3)_2 \cdot 6H_2O$
11. $Ca(NO_3)_2$
12. $Sr(NO_3)_2$

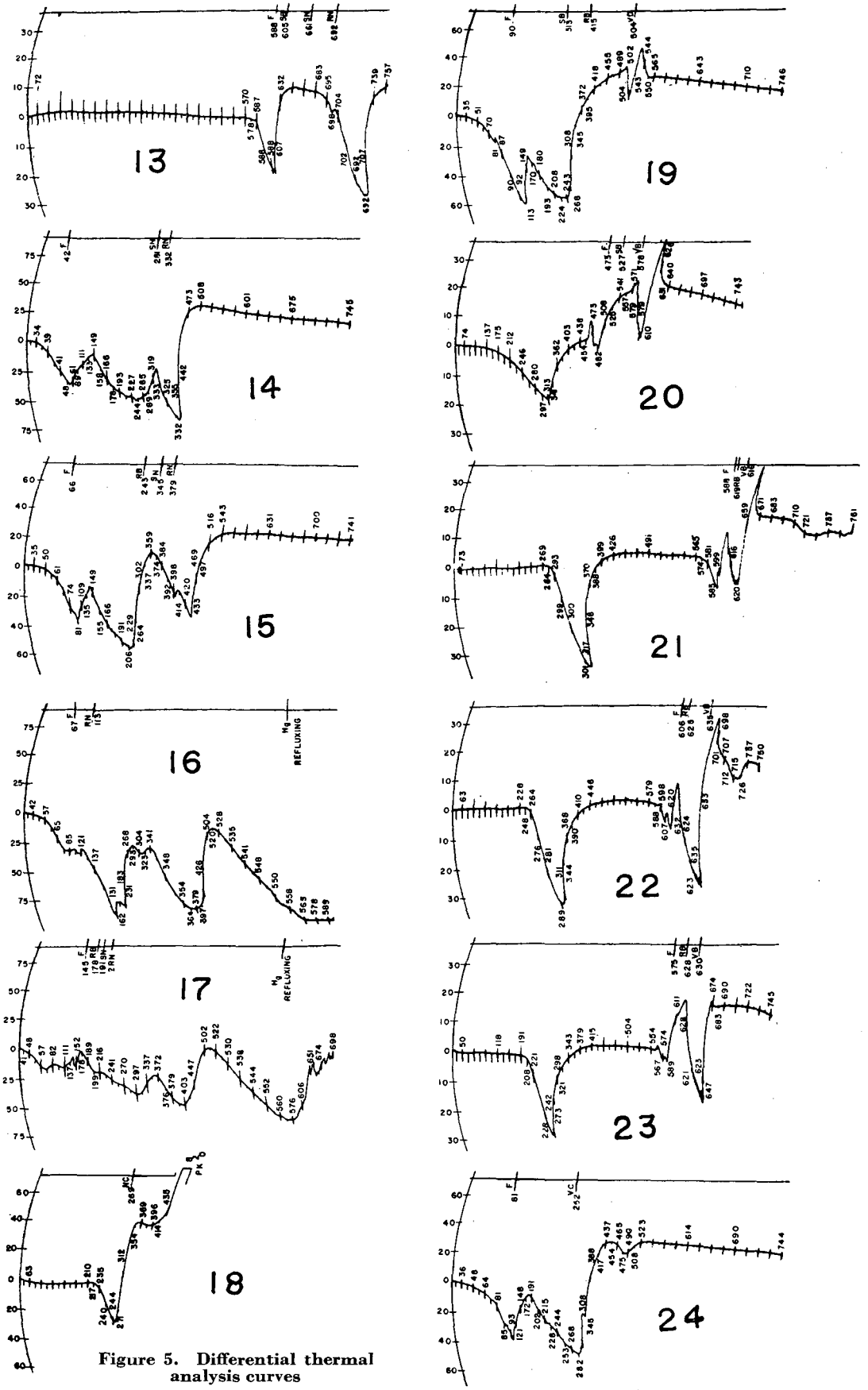

Figure 5. Differential thermal analysis curves

13. $Ba(NO_3)_2$
14. $Zn(NO_3)_2.6H_2O$
15. $Cd(NO_3)_2.4H_2O$
16. $HgNO_3.H_2O$
17. $Hg(NO_3)_2.{}^1/_2H_2O$
18. NH_4ClO_4
19. $LiClO_4.3H_2O$
20. $NaClO_4$
21. $KClO_4$
22. $RbClO_4$
23. $CsClO_4$
24. $Cu(ClO_4)_2.6H_2O$

were obtained as a function of time on the Brown and GE recorders, respectively, at chart speeds of 6 inches per hour. In order to synchronize and correlate the temperature parameter of the differential thermal analysis curve, with that of the sample temperature curve, it was necessary to place synchronous timing marks on the two records (*5*). This was accomplished by means of a synchronous motor-driven recycling multicam timer (Industrial Timer Corp., CM-5) with microswitches which were incorporated into the circuits of the sample thermocouple for the Brown recorder and the lamp filament circuit of the GE recorder. A representative pair of heating and differential temperature curves for potassium perchlorate are shown in Figure 3. The temperatures at the various 1-minute intervals are readily transferred to the differential temperature curve. Temperatures corresponding to the large and small marks are indicated above and below the curve, respectively. In addition, the Brown and GE recorder chart drive motors and the synchronous timing motor were started simultaneously by the controlling mercury switch in the Brown recorder at any predetermined temperature, most often 40° C.

On the reference line above the curve are auxiliary pen marks denoting the temperatures and times at which visible reactions such as fusion, boiling, decomposition, etc., occurred. A legend for the symbols employed is presented in Table I.

The furnace (*B*, Figure 1), controlled by a 15-ampere Powerstat at 135 volts, had a heating rate of about 15° C. per minute over the range of 60° to 600° C. above which the rate decreased slightly. The maximum temperature employed was 920° C., although most determinations were terminated at 740° to 760° C. Above these temperatures there was a tendency for the borosilicate glass test tubes to fuse with the steel block, thereby rendering the block useless for further studies. Consequently, very few samples were heated beyond this temperature range. The low cost of the culture-type test tubes permitted them to be used expendably, so that each sample was run in a new reaction tube. Test tubes of 96% silica permitted analyses up to the highest temperature limit of the furnace (about 1000° C.), but the decreased heating rate detracted from the value of these runs. By using two identical blocks and furnaces, it was possible to perform another differential thermal analysis while the previously heated block and furnace were cooled in water and a stream of compressed air, respectively.

EFFECTS OF EXPERIMENTAL VARIABLES

A preliminary study of the factors which might influence the differential thermal analysis curves was made before the technique described above was established. It was found that the quantity of sample used was conveniently contained in a standard size test tube and gave temperature differentials which could be readily recorded with the afore-mentioned instrumentation. Furthermore, this size of sample produced thermal phenomena which were easily observed, yet insufficiently large to present a hazard during any violent reactions. The length of the test tube employed permitted the contents to be placed down in the center of the furnace well with sufficient tube above the furnace to prevent the loss of sample during expulsive reactions and presented a cool surface for the formation and observation of condensates. It was practical to use a new tube for each sample, thereby eliminating the presence of any contaminating residues which might affect subsequent determinations. The glass thermocouple protection wells were selected for this same reason.

All samples investigated were ground with a mortar and pestle to average particle diameters of 50 to 150 microns. This particle size is well within that recommended by Grim (*6*) for obtaining reproducible densities for the samples in the reaction tubes, which is necessary for duplication of the differential thermal analysis curves. To ascertain the effect that extremes of particle sizes would have on the curves, potassium perchlorate was run as received (coarse, granular crystals) and after size reduction to 30 microns. The respective curves were found to have the same bands, "pips," and sample temperature inflections.

The curves presented were obtained with the GE photoelectric strip-chart recorder (*4*). The recording mechanism of this pen-and-ink instrument is a permanent-magnet, moving-coil, pen motor which causes the deflections to be drawn as arcs rather than straight lines. The differential temperatures are plotted against the sample temperature, rather than that of the reference material or the furnace in order to indicate the temperatures at which the thermal phenomena occur within the compound under investigation. The sample temperature axes are nonlinear because the differential and sample temperatures are recorded as a function of time on their respective instruments, and the values are transferred on a basis of the timing marks as previously described.

The "base line" of a differential thermal analysis curve does not always correspond to a zero temperature differential because the thermal diffusivity of the sample with respect to the reference compound often varies with temperature, or as a result of transitions, changes of state, or the formation of new products from dehydration or decomposition reactions. Consequently, the new base line may be displaced above or below the zero line. In this paper the area above this zero differential reference line is referred to as the exothermal region and that area below this line as the endothermal region. The occasional "endothermal dip" (Figures 4 to 6, curves 3, 4, 21, 26, 29, 31) present between 700° and 740° C. in the exothermal region occurs in the temperature range at which furnace coil resistance wires—e.g., Nichrome (*3*)—exhibit a characteristic inversion of the resistance coefficient which then levels off before again increasing. This causes the heating rate of the furnace to decrease noticeably as evidenced by the sample temperature curve. The block, which is then at a relatively constant temperature for a short time, is hotter than the reference compound and the sample under study. Consequently, the block transfers heat to the cooler tubes and contents so as to equilibrate the system thermally; the reference tube, heating more rapidly because it is at a lower temperature, therefore causes an "apparent endotherm" to appear as the differential temperature departs from the previously established base line in this region where no thermal reactions are occurring. When,

Table I. Index to Differential Thermal Analysis Curves, Temperatures of Fusion, and Visible Decomposition Phenomena

Curve No.	Formula[a]	Fusion, °C.[b]	Decomposition Phenomena, °C.[c]
1	NH_4NO_3	170	RB-249, VB-289, RN-395
2	$LiNO_3$	252	SB-365, RB-477, SN-612, RN-635
3	$NaNO_3$	304	SB-520, RB-612, SN-710, RN-753, VN-777
4	KNO_3	332	SB-628, RB-642, SN-805
5	$RbNO_3$	294	SB-609, RB-642, VB-881
6	$CsNO_3$	404	SB-531, RB-655, VB-850, SN-849
7	$Cu(NO_3)_2.3H_2O$	120	RN-203
8	$AgNO_3$	214	RB-305, RN-469
9	$Be(NO_3)_2.3H_2O$	74	SN-153, RN-220
10	$Mg(NO_3)_2.6H_2O$	107	SN-321, VN-430
11	$Ca(NO_3)_2$	550	RB-581, RN-612
12	$Sr(NO_3)_2$	618	VB-672, SN-635, RN-715
13	$Ba(NO_3)_2$	588	SB-605, SN-661, RN-692
14	$Zn(NO_3)_2.6H_2O$	42	SN-281, RN-332
15	$Cd(NO_3)_2.4H_2O$	66	SN-345, RN-379
16	$HgNO_3.H_2O$	67	RN-113
17	$Hg(NO_3)_2.{}^1/_2H_2O$	145	RB-178, SN-191, RN-212
18	NH_4ClO_4		NC-269, PK-820
19	$LiClO_4.3H_2O$	90	SB-310, RB-412, VB-504
20	$NaClO_4$	473	SB-527, VB-578
21	$KClO_4$	588	RB-619, VB-616
22	$RbClO_4$	606	RB-625, VB-635
23	$CsClO_4$	575	RB-628, VB-630
24	$Cu(ClO_4)_2.6H_2O$	81	RC-252
25	$AgClO_4$		D-277, VB-473
26	$Mg(ClO_4)_2$	246	SB-334, VB-367
27	$Ca(ClO_4)_2$	123	SB-258, VB-285
28	$Sr(ClO_4)_2$		VD-477
29	$Ba(ClO_4)_2$	469	VD-504
30	$Zn(ClO_4)_2.6H_2O$	170	RC-260
31	$Hg(ClO_4)_2.3H_2O$	38	RC-212

[a] Hydrates determined without drying; all others dried at 110° C. except $Ca(NO_3)_2$ (150° C.).
[b] Temperature at which mass is observed to undergo fusion or hydrate dissolution.
[c] F fusion, SB slight bubbling, RB rapid bubbling, VB vigorous bubbling, SN slight nitrous fumes (brown NO_2), RN rapid nitrous fumes, VN vigorous nitrous fumes, C chlorous fumes (white, chlorinelike odor), SC slight chlorous fumes, RC rapid chlorous fumes, NC nitrous and chlorous fumes, VD vigorous decomposition, D color change decomposition, PK temperature at peak of exotherm.

Table II. Tabulation of Crystalline Transitions

Formula	Endotherm (DTA), °C.	DTA Transition Temp., °C.[a]	Reported Transition Temp., °C.	Crystalline Modifications
NH_4NO_3	35– 50	33	32	Rhombic II → rhombic I
	76–101	89	84	Rhombic I → tetragonal
	117–141	129	125	Tetragonal → cubic
$NaNO_3$	206–273	273	275	Rhombic → trigonal
KNO_3	114–139	128	128	Rhombic → trigonal
$RbNO_3$	153–170	167	161	Hexagonal → cubic
	215–227	221	219	Cubic → rhombic
$CsNO_3$	145–164	160	161	Hexagonal → cubic
$AgNO_3$	154–181	169	160	Rhombic
NH_4ClO_4	235–244	240	240	Rhombic → cubic
$NaClO_4$	175–313	313	308	Rhombic → cubic
$KClO_4$	284–301	300	300	Rhombic → cubic
$RbClO_4$	264–289	281	279	Rhombic → cubic
$CsClO_4$	208–242	224	219	Rhombic → cubic
$AgClO_4$	143–(<174, >157)	157	155–159	Rhombic → cubic
	174–185	175		

[a] See text for description of estimation technique.

in this temperature range, either the base line is near the zero differential line or the thermal diffusivities of the sample (or its decomposition products) and the reference compound are comparable, there is a more symmetrical temperature equilibration and the base line remains constant.

In the study of pyrotechnic compositions, which react very rapidly and create extremely large temperature gradients, the oxidants are subjected to rapid heating rates. Consequently a heating rate of approximately 15° C. per minute, the maximum constant rate that could be attained with the resistance furnaces employed, was used.

DISCUSSION OF RESULTS

The differential thermal analysis curves shown in Figures 4 to 6 are indexed in Table I, with a summary of the apparent physicochemical phenomena exhibited by the compounds and the temperatures at which they are observed during the differential thermal analyses. The estimated and reported transition temperatures (*1*, *9*, *11*) of the compounds which undergo crystalline transformations are summarized in Table II.

The curves presented resemble the spectra obtained in absorption spectrophotometry, in that the positions, magnitudes, and profiles of the bands and peaks serve to identify specific physicochemical properties characteristic of the respective compounds; they represent the many unique thermal parameters that can be measured and quantitatively recorded with the previously described instrumentation. Alterations in the size, subdivision, and packing of the sample, type of sample tube, heating rate, geometry of the apparatus and the type of recording system will influence the appearance of the differential thermal analysis curve for any given system. However, when these parameters of the differential thermal analysis system are held constant, quantitative significance may be assigned to the relative amplitudes and positions of the many bands, peaks, and pips which constitute the patterns of the curves obtained.

The relatively large samples and furnace blocks employed in this study result in a system with inherently larger thermal lags (response time to temperature changes) than would be the case

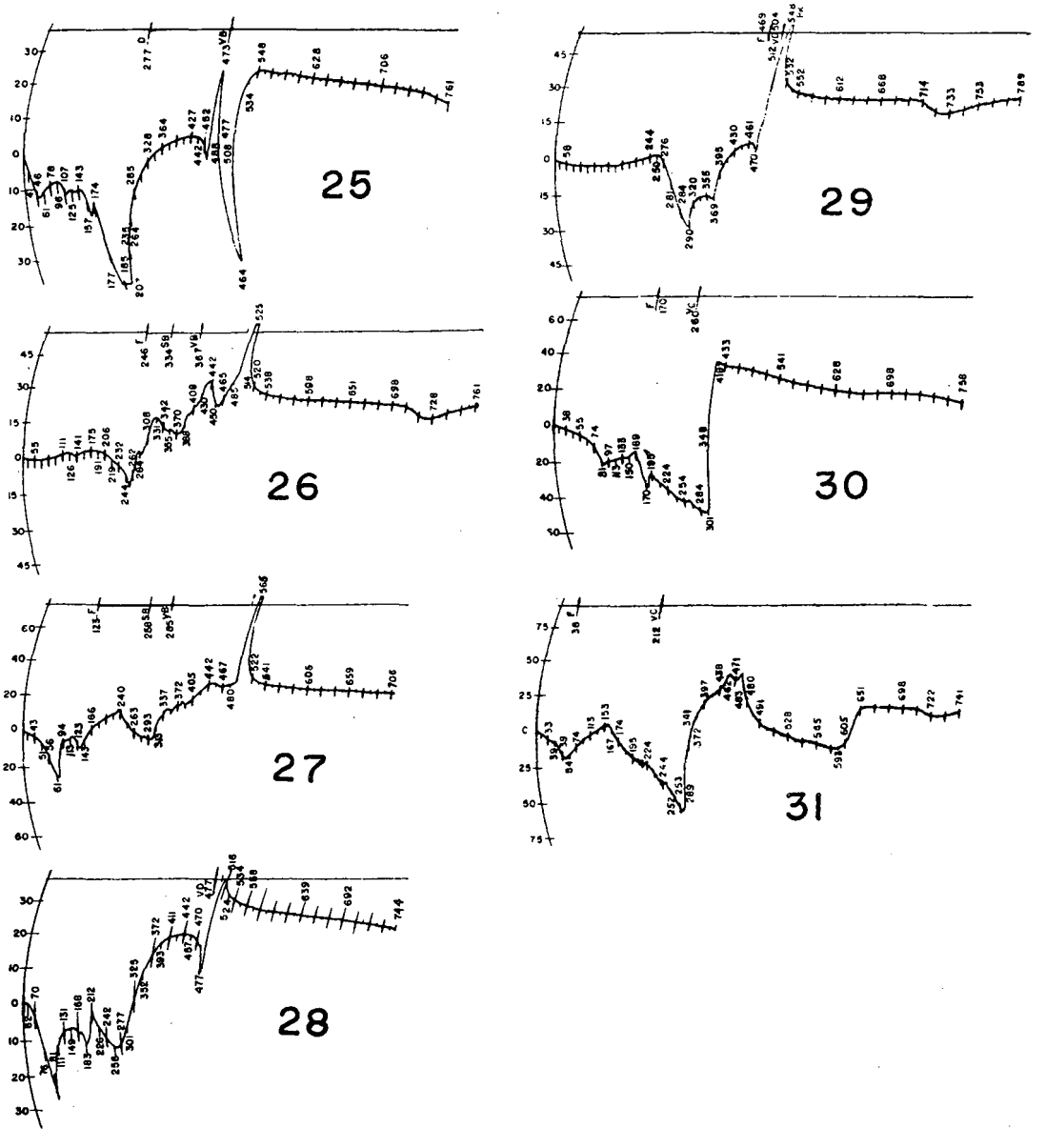

Figure 6. Differential thermal analysis curves

25. $AgClO_4$
26. $Mg(ClO_4)_2$
27. $Ca(ClO_4)_2$
28. $Sr(ClO_4)_2$
29. $Ba(ClO_4)_2$
30. $Zn(ClO_4)_2.6H_2O$
31. $Hg(ClO_4)_2.3H_2O$

for a considerably smaller one. When the sample in the outer annular portion of the tube reaches a temperature at which there is some thermal phenomenon, the thermocouple in the center of the sample will measure a lower temperature. Therefore, the time and temperature at which a differential inflection begins will be premature with respect to the central mass. However, the "true" temperature of the thermal phenomenon generally lies close to the temperature corresponding to the differential peak, since at this point the bulk of the sample is undergoing the final stages of the respective change.

The transition points were obtained from the differential thermal analysis curves by means of the temperatures corresponding to the consecutive 1-minute timing marks, from a point before the initial endothermal inflection to the point following the endothermal peak. The rate of change of sample temperature was used as the basis for determining these temperatures. The transition points were chosen as either the temperature at the timing mark lying between the two smallest temperature changes for successive 1-minute intervals or the mid-point of the interval having the smallest temperature change, when the intervals to either side were approximately equal to each other but larger than the smallest one. The values for the sodium nitrate and perchlorate transition points were taken as the temperature at the last reference mark prior to the large temperature interval following the endothermal peak. This was done because of the relatively constant heating rate which accompanies the slow transition over a fairly large temperature range and is further explained under the discussion of the alkali metal nitrates and perchlorates. The values obtained from the curves, although in most cases slightly higher than those reported in the literature, were in good agreement—i.e., within 5° C.

NITRATES

The alkali metal and alkaline earth nitrates appear to undergo thermal decomposition according to the same general pattern, progressively exhibiting many of the following physicochemical phenomena on the differential thermal analysis curves: crystalline transformation, dehydration (if hydrated), melting, bubbling, and a final stage of rapid decomposition accompanied by a pronounced endotherm. All of the alkali metal nitrates with the exception of lithium exhibited crystalline transitions (Table II and Figure 4, curves 2 to 6). The relatively broad transition endotherm for sodium nitrate results from the gradual rotation of the nitrate ion during the transformation of the rhombic to the trigonal crystal modification as the temperature increases. In potassium nitrate, however, the transformation corresponding to the endotherm starting at 114° C. takes place rather sharply at about 128° C. and the break in the differential thermal analysis curve is steep. Rubidium nitrate is the only alkali metal nitrate that undergoes two transitions. The alkali metal nitrates undergo a thermal reaction at temperatures 100° to 300° C. above their melting points as indicated by bubbling of the molten mass. The evolution of nitrous fumes which occurs at temperatures ranging from about 200° to 350° C. above the initial bubbling reaction, is a result of the decomposition of the nitrate and/or nitrite to oxide. Furthermore, the latter stage of the decomposition of these nitrates is still occurring at temperatures as high as 900° C., the upper limit of the apparatus employed in this investigation.

The alkaline earth nitrates (Figures 4, and 5, curves 9 to 13), unlike the alkali metal salts, undergo no crystalline transitions. In this respect they apparently resemble lithium—the only member of the latter group which does not exhibit transformation. Beryllium and magnesium, the first two members of the alkaline earth group, were necessarily determined as hydrates, inasmuch as their instability and extreme hygroscopicity withstood attempts to dehydrate them. These hydrated salts fuse, or dissolve in their water of hydration, and decompose with the evolution of nitrous fumes at relatively low temperatures without the intermediate colorless bubbling reaction. Prior to the evolution of nitrous fumes, these liquid hydrated nitrates decompose in such a way as to generate nitric acid vapors. The latter three alkaline earths—calcium, strontium, and barium—undergo a bubbling type of visible reaction at a temperature less than 50° C. above their melting points, and decompose to the oxide and nitrous fumes at temperatures about 50° C. above the initial temperature of visible ebullition. An interesting phenomenon characteristic of these alkaline earth nitrates is the magnitude of the endothermal dip accompanying the rapid evolution of nitrous fumes. For some compounds (Figures 4 and 5, curves 10 and 13) this endotherm is so pronounced that the temperature of the sample actually decreases for a short period of time, despite the constantly increasing temperature of the furnace.

Ammonium nitrate (Figure 4, curve 1), which has three transition points, fuses at about 170° C. and decomposes visibly at a temperature less than 100° C. above this point. The bubbling decomposition becomes endothermal at about 290° C., and at 325° C. the tube contents have been almost completely exhausted because of the volatile decomposition products formed. The Group IB nitrates, copper and silver (Figure 4, curves 7 and 8), were thermally analyzed as trihydrated and anhydrous salts, respectively. The thermal decomposition of the hydrated copper (II) salt involves three endotherms with no further visible reaction beyond about 400° C. Fusion, or dissolution, occurs at 120° C. and the initial appearance of nitrous fumes at about 200° C. The silver nitrate curve exhibits three endotherms, the first of which corresponds to the crystalline transition (*1*). The fusion endotherm occurs at 214° C., followed by a bubbling at 305° C., and a rapid nitrous fume evolution takes place at 469° C. which is accompanied by a large endotherm ending at 650° C.

The Group IIB nitrates—zinc, cadmium, and mercury(I and II) (Figure 5, curves 14 to 17)—which were all hydrated, fused by dissolution at increasingly higher temperatures (42° to 145° C.). The first three of these compounds evidenced visible decomposition by the formation of nitrous fumes, whereas the mercury (II) nitrate bubbled rapidly and subsequently evolved the nitrogen oxides. The zinc nitrate exhibits three endotherms: The first seems to be due to fusion (dissolution), the second is accompanied by nitric acid fumes, and the last occurs during the rapid evolution of nitrous fumes, the thermal reaction being completed at about 470° C. Similarly, the cadmium nitrate curve shows three corresponding endotherms with the decomposition thermally complete at about 540° C. The latter curve has an additional small pip on the third endotherm. The mercury (I) and mercury (II) nitrate curves exhibited many endotherms, the last one being that for the refluxing mercury, a final decomposition product apparently resulting from the decomposition of mercury (II) oxide—the two curves having similar shapes beyond 350° C.

PERCHLORATES

The alkali metal perchlorates (Figure 5, curves 19 to 23), with the exception of lithium, undergo transitions from the rhombic to the cubic lattice, the temperature of transition decreasing with increasing atomic number. The transition in sodium perchlorate, as in the nitrate, appears to be gradual, while that of the potassium salt occurs sharply. Lithium perchlorate was determined as the hydrate because of the extreme deliquescence of the anhydrous material. The rapid decomposition of the alkali metal perchlorates occurs immediately after melting and appears as a sharp exothermal deflection. This exothermal decomposition is characteristic of all the perchlorates of Groups I and II.

The decomposition pattern of the alkali metal perchlorates, consisting of two endothermal peaks in the curve, is an effect of sample size. Apparently, the material in the outermost layer, next to the tube wall, melts (endothermal) and begins to decompose (exothermal) before the central mass near the thermocouple

has melted. The competing endothermal and exothermal reactions result in the dual break in the curve. By decreasing the sample size the second endotherm is minimized and finally eliminated. Some of these decompositions were sufficiently vigorous in their bubbling action to cause fluctuations in the sample temperature so that during the course of this reaction at progressively higher furnace temperatures the sample temperature increases and decreases in a seemingly erratic manner.

The endotherms due to the melting points of the respective chlorides formed by the decomposition may be seen in the differential thermal analysis curves for rubidium and cesium perchlorates. Corresponding points for sodium and potassium perchlorates lie above the upper temperature of the thermal analysis and do not appear on the curves.

The alkaline earth perchlorates (Figure 6, curves 26 to 29), as in the case of the nitrates, resemble the lithium salt since they do not undergo crystalline transition. Beryllium perchlorate was not included in this study because it is not commercially available and the need did not warrant the development of a method for its synthesis. Since all of these perchlorates are widely used as powerful desiccants because of their extreme deliquescence, it was virtually impossible to dry and pulverize the samples for the thermal analyses without their absorbing moisture during handling in the laboratory atmosphere. The moisture content of these samples is reflected in the low temperature endotherms prior to fusion and/or decomposition. The multiplicity of the endothermal bands of the strontium and barium salts is not completely understood, although some can be accounted for by the accompanying visible phenomena, such as fusion and decomposition. The other bands which occur below the first visible signs of exothermal reaction, but at relatively high temperatures for dehydration, may be the result of dehydration or some unreported crystalline transitions.

Magnesium and calcium perchlorates, which pass through progressively more rapid stages of bubbling decomposition (commencing 90° and 130° C. above their melting points, respectively), decompose very exothermally at temperatures of 525° and 565° C., respectively. The strontium and barium salts show no evidence of visible reaction until their vigorous decomposition, which occurs concurrently with their exothermal bands. The barium perchlorate fuses at the onset of this exothermicity.

Ammonium perchlorate (Figure 5, curve 18) decomposes with the evolution of nitrous and chlorous fumes shortly after transition from the rhombic to the cubic lattice. At about 435° C., there is an extremely rapid exothermal decomposition, resulting in a recorded peak temperature (*PK*) of 820° C. This value is appreciably lower than the true temperature since the thermocouple-recorder lag time precludes a sufficiently rapid response to follow the temperature rise of the system. After this reaction the sample tube is empty because of the volatility of the reaction products formed. The exothermicity of this decomposition was by far the greatest obtained with any of the nitrates or perchlorates studied by differential thermal analysis.

Copper(II) perchlorate hexahydrate (Figure 5, curve 24) fused by dissolution below 100° C. and evolved chlorous fumes at about 250° C., these phenomena being accompanied by rather broad endotherms. A third endotherm, at about 475° C., corresponds to the fusion of copper(II) chloride. Silver perchlorate (Figure 6, curve 25), although oven dried, may have absorbed moisture which would account for the low temperature endotherms prior to crystalline transition. The transition is followed by a decomposition evidenced in a color change (white to brown), then a small endotherm, followed by a large one accompanying a vigorous bubbling of the system. The thermal reactions are completed at about 550° C. The small endotherm may be due to the fusion of some silver chloride formed by the initial stages of decomposition.

Cadmium and mercury(I) perchlorates are not commercially available and were omitted from this study. Zinc and mercury(II) perchlorates (Figure 6, curves 30 and 31), hexa- and trihydrates, respectively, exhibited rather ill-defined endotherms. The zinc curve has a low temperature endotherm, which may be dehydration, a fusion band at 170° C., and exothermal chlorous fume decomposition starting at 260° C. The thermal reaction is completed at 420° C. Mercury(II) perchlorate has a dissolution endotherm at 38° C., a broad endotherm accompanied by vigorous chlorous fumes from 150° to 250° C., and then a complex band for the latter stages of decomposition to metallic mercury.

Further investigations of the thermal behavior and decomposition phenomena associated with many of the above compounds are in progress. Particular emphasis is to be placed upon defining and interpreting many of the endotherms and visible reactions reported in this paper, for which no satisfactory explanations are known to the authors.

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Determination of Olefins by Means of Iodine Complexes

Ultraviolet Absorption Method

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This paper describes a procedure for distinguishing types of alkyl-substituted olefins by means of the ultraviolet spectra of reversible complexes formed with iodine. Very intense bands are observed, as follows: RCH=CH_2, 275 mμ; R_2C=CH_2, 290 to 295 mμ; RCH=CHR, 295 to 300 mμ; R_2C=CHR, 317 mμ; and R_2C=CR_2, 337 mμ. Cyclopentene and cyclohexene behave like the cis form of the corresponding open-chain olefin RCH=CHR. The method is of particular interest for the determination of the tri- and tetrasubstituted olefins because these types are difficult to determine by infrared or Raman spectroscopy. Olefin (0.03 to 0.1 mole per liter) and iodine (0.002 mole per liter) are dissolved in iso-octane, and the ultraviolet spectrum of the complex is measured in a cell of 1-cm. path length. For these concentrations it is shown that a simple extension of the Beer's law equation applies in which the measured absorbance is proportional to the product of the olefin and iodine concentrations. Apparent molecular "absorptivities" can thus be calculated and are found to be reasonably constant for a given olefin type.

A NUMBER of workers (*2, 3, 5, 6*) have shown that iodine forms reversible complexes with a variety of organic molecules such as ethers, alcohols, ketones, sulfides, olefins, diolefins, aromatics, and even paraffins. In particular, the iodine-olefin complexes have a very intense band in the 275- to 350-mμ region of the ultraviolet spectrum. It is shown here that mono-, di-, tri-, and tetrasubstituted ethylenes can be determined because the band for each type occurs at a characteristic wave length (Figure 1).

The most widely used method for olefin-type analysis depends on infrared absorption bands due to ethylenic hydrogen vibrations, which occur in the 800- to 1000-cm.$^{-1}$ region (*1, 9*). This method is good for the simpler olefin types, RCH=CH_2, R_2C=CH_2, and *trans*-RCH=CHR. For these olefins, the characteristic band for each type falls within a very narrow absorption region, regardless of the nature of the *R* group. However, the trisubstituted olefins, R_2C=CHR, have an absorption maximum which may occur over the whole 800- to 850-cm.$^{-1}$ region. Since the exact position of the absorption band for this olefin type depends strongly on the nature of the R groups, the analysis is unsatisfactory. Finally, the fully substituted olefin, R_2C=CR_2, has no ethylenic hydrogens and therefore no infrared bands of this type are even available for analysis.

Raman spectra can also be used to distinguish the various olefin types by means of the C=C stretching frequencies in 1650 to 1680-cm.$^{-1}$ region (*4*). Although all of the olefin types have characteristic Raman bands, the *trans*-RCH=CHR, R_2C=CHR, and R_2C=CR_2 types cannot be distinguished because their bands substantially overlap.

Analysis for olefin types by ultraviolet spectroscopy is possible in the "vacuum region" below 2000 A. (*3*). The simpler olefins have bands in this region, and increasing alkyl substitution shifts the absorption maxima toward longer wave lengths. Unfortunately, the experimental technique is difficult, and few laboratories are equipped with the necessary vacuum spectrometers.

The iodine-olefin complex procedure described here is useful for all of the olefin types, and the work can be done with ordinary ultraviolet spectrometers. The method is of particular interest because the tri- and tetrasubstituted olefins can be distinguished.

PROCEDURE

Summary of Method. The experimental procedure is comparatively simple. Olefin (0.05 mole per liter) and iodine (0.002 mole per liter) are dissolved in purified iso-octane. The spectrum is then recorded from 400 to 250 mμ with a Cary spectrometer (Model 11) and an absorption cell of 1-cm. path length.

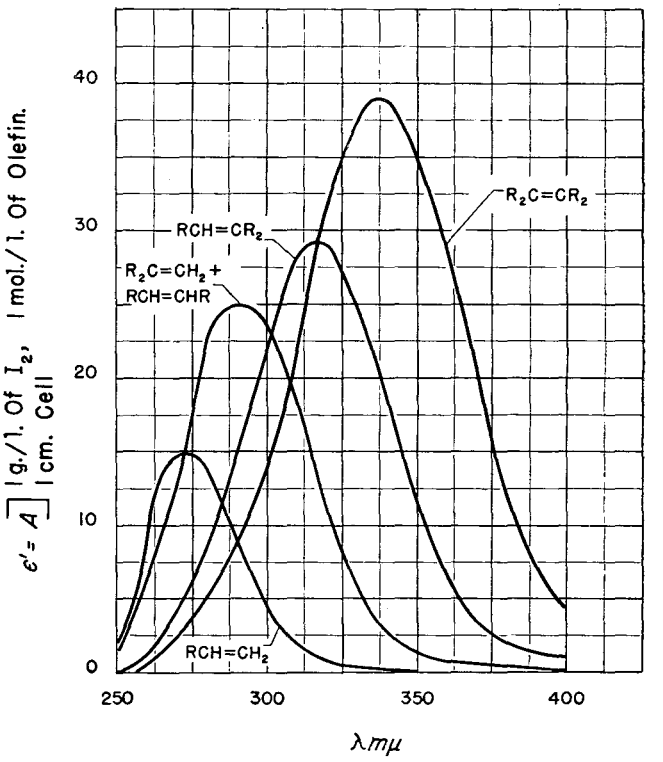

Figure 1. Olefin-iodine complexes for various olefin types

Reagents. Iso-octane of 99% purity (Phillips pure grade) is used as the solvent. The iso-octane is purified by shaking it thoroughly with concentrated sulfuric acid. A liter of the acid-washed iso-octane is then percolated through a 3-foot column of 1-inch diameter filled with 200-mesh silica gel (Davison Chemical, Code 950). This treatment removes traces of impurities such as aromatics and dienes. The solvent should have an absorbance less than 0.05 down to 230 mμ, when compared to distilled water in a cell of 1-cm. path length.

The iodine reagent is a solution of iodine crystals in the purified iso-octane, at a concentration of 1 gram per liter. Approximately 0.1 gram of iodine, weighed to the nearest 0.1 mg., is dissolved and diluted to 100 ml. in the iso-octane.

Calibration. API (American Petroleum Institute) standard samples of olefins are recommended for calibration. A dilution of the olefin is prepared at a known concentration of approximately 0.1 mole per liter in the purified iso-octane. Five milliliters of the prepared olefin solution are then added to 5 ml. of the iodine reagent. The spectrum of the resulting solution is meas-

ured from 400 to 250 mμ, using a silica absorption cell of 1-cm. path length. The solution is measured immediately after mixing to avoid the catalytic effect of light on the formation of undesired diiodo compounds.

To keep the absorbance of the complex between 0.3 and 1.0, it may be necessary to vary the olefin concentration. If so, a second dilution of the olefin in the iso-octane is prepared, and then the iodine reagent is added. Mere dilution of the final iodine-olefin solution with solvent is not satisfactory, since the iodine concentration should not be varied.

A correction for background due to uncomplexed iodine is obtained by adding 5 ml. of the iodine reagent to 5 ml. of iso-octane and then measuring the solution as described above. No background correction for the uncomplexed olefin is made in the calibration procedure because the pure olefins are transparent in this region.

Measurements reported in this paper are made at 25° ± 1° C. As the Cary spectrometer used does not warm the solutions appreciably and the ΔH of the iodine-olefin complexes is small [ca. −500 cal. per mole (*5*)], no significant temperature effects are to be expected.

To obtain calibration absorptivities, measurements are made at the wave lengths of maximum absorption ($\lambda_{max.}$) characteristic of each olefin type, as follows (see Figure 1):

Olefin Type	$\lambda_{max.}$, mμ
$RCH{=}CH_2$	275
$R_2C{=}CH_2$	290–295
$RCH{=}CHR$	295–300
$R_2C{=}CHR$	317
$R_2C{=}CR_2$	337

The measured absorbances are first corrected for the iodine background absorption. "Molecular absorptivities" are then calculated as follows:

$$\epsilon' = \frac{A_c}{(C_I) \times [C_O]}$$

where

ϵ' = molecular absorptivity
A_c = absorbance at $\lambda_{max.}$, corrected for I_2 background
(C_I) = concentration of I_2 in the final solution, in grams per liter
$[C_O]$ = concentration of olefin in the final solution, in moles per liter

Molecular absorptivities at the appropriate $\lambda_{max.}$ for a number of olefins are given in Table I. For a multicomponent analysis, overlap absorptivities are obtained in a similar manner at each of the wave lengths used.

For ordinary quantitative analysis, as distinguished from type analysis, it is preferable to calculate a "weight absorptivity," a', defined as follows:

$$a' = \frac{A_c}{(C_I) \times (C_O)}$$

where

a' = weight absorptivity
A_c = absorbance at $\lambda_{max.}$, corrected for I_2 background
(C_I) = concentration of I_2 in final solution, grams/liter
(C_O) = concentration of olefin in the final solution, grams/liter

DISCUSSION

Basic Theory. In order to understand the choice of concentrations and the method of expressing absorptivities, it is necessary to examine briefly the underlying theory of iodine complex formation. It is known that iodine and hydrocarbons undergo a reversible reaction to form an addition complex of an acid-base type (*3*, *7*). For olefins the equation is:

$$I_2 + \text{olefin} \leftrightharpoons I_2 \times \text{olefin} \cdot [\Delta H \text{ ca. } -500 \text{ cal. per mole } (5)]$$

The iodine functions as an electron-acceptor (Lewis acid), and the hydrocarbon functions as the electron-donor (Lewis base). At equilibrium, the concentrations of the complex and the equilibrium constant, K, are defined by the mass law equation:

$$K = \frac{[I_2 \times \text{olefin}]}{[\text{olefin}] \times [I_2]}$$

The particular problem in the analytical application of iodine complexes is that the equilibrium constant, K, the true molar absorptivity, ϵ, of the complex, and the concentration of the complex cannot readily be determined with precision. Benesi and Hildebrand (*3*) have described a mathematical procedure for deriving these quantities from absorbance measurements on a series of solutions. However, this is exacting and tedious work, and will be shown to be unnecessary for analytical applications.

For analytical purposes it is essential that the absorbance measured be directly proportional to the concentration of olefin in the final solution. In order to choose concentrations of iodine and olefin to meet this requirement, an approximate value for the equilibrium constant must be known. It has been shown that the true molar absorptivity, ϵ, of iodine complexes falls within the rather narrow range from 10,000 to 25,000 liters per mole-cm. for such diverse molecules as aromatic, cycloparaffin, and paraffin hydrocarbons, and even for an alkyl halide (*3*, *7*). Therefore, if

Table I. Molecular Absorptivities for Iodine-Olefin Complexes

Olefin Type	Compound	I_2 Concn., Gram/Liter	Olefin Concn., Mole/Liter	$\lambda_{max.}$, Mμ	A, 1-Cm. Cell	ϵ', 1 Mole/Liter of Olefin, 1 Gram/Liter of I_2
$R_2C{=}CR_2$	2,3-Me_2-2-butene	0.4405	0.0636	337	1.086	38.7
	2,3-Me_2-2-pentene	0.5105	0.0453	337	0.711	30.8
	2,3-Me_2-2-hexene	0.5105	0.0616	337	0.937	29.8
$RCH{=}CR_2$	2-Me-2-butene	0.5040	0.0773	313	1.090	28.0
	3-Me-*trans*-2-pentene	0.5040	0.0795	317	1.089	27.1
	3-Me-*cis*-2-pentene	0.4405	0.0647	317	0.825	28.9
	2-Me-2-pentene	0.5470	0.0761	317	1.180	28.4
	2-Me-2-hexene	0.4405	0.0703	317	0.804	25.9
	3-Me-*cis*-3-hexene	0.5280	0.0465	317	0.648	26.4
	3-Et-2-pentene	0.6840	0.0477	317	0.790	24.2
$R_2C{=}CH_2$	2,3-Me_2-1-butene	0.6840	0.0389	293	0.634	23.8
	2-Me-1-pentene	0.5470	0.0817	293	1.079	24.1
	2-Et-1-butene	0.6840	0.0354	290	0.862	32.5
	2-Me-1-hexene	0.5280	0.0815	293	1.090	25.3
	2,3,3-Me_3-1-butene	0.5470	0.0654	295	0.918	25.7
$RHC{=}CHR$	*cis*-2-Pentene	0.6840	0.0795	293	1.134	20.9
	trans-2-Pentene	0.5105	0.0999	295	0.692	13.6
	cis-2-Hexene	0.6765	0.0689	294	0.915	19.7
	trans-2-Hexene	0.6765	0.0700	295	0.618	13.1
	cis-3-Hexene	0.5000	0.0855	295	0.884	20.7
	trans-3-Hexene	0.5000	0.0832	295	0.567	13.7
	4-Me-*cis*-2-pentene	0.5470	0.0923	295	0.682	16.2
	4-Me-*trans*-2-pentene	0.5105	0.0898	297	0.511	11.2
	4,4-Me_2-*cis*-2-pentene	0.6840	0.0704	295	0.788	16.4
	4,4-Me_2-*trans*-2-pentene	0.6840	0.0713	295	0.370	7.6
	trans-2-Heptene	0.5075	0.0767	297	0.524	13.5
	trans-3-Heptene	0.5280	0.0845	300	0.531	11.9
	trans-4-Octene	0.5470	0.0803	300	0.481	10.9
	2,2-Me_2-*cis*-3-hexene	0.5280	0.0760	300	0.683	17.1
	2,2-Me_2-*trans*-3-hexene	0.5280	0.0736	300	0.266	6.8
$RHC{=}CHR$ cyclic	Cyclopentene	0.5095	0.0690	300	0.732	20.8
	Cyclohexene	0.5095	0.0456	295	0.667	29.2
$RCH{=}CH_2$	1-Hexene	0.5040	0.0733	275	0.449	12.1
	3,3-Me_2-1-butene	0.5470	0.0668	275	0.428	11.7
	3-Me-1-pentene	0.5280	0.0972	275	0.505	9.9
	4-Me-1-pentene	0.5470	0.0713	275	0.393	10.1
	1-Heptene	0.5365	0.0693	275	0.480	12.9
	4-Me-1-hexene	0.5280	0.0906	275	0.489	10.2
	5-Me-1-hexene	0.5280	0.0853	275	0.558	12.4
	4,4-Me_2-1-pentene	0.5365	0.0773	275	0.368	8.9
	1-Octene	0.6840	0.0623	275	0.553	13.0
	1-Nonene	0.5365	0.0671	275	0.460	12.8
	1-Decene	0.5105	0.0626	275	0.424	13.3
	1-Dodecene	0.5075	0.0577	275	0.410	13.9
	1-Tetradecene	0.5365	0.0645	275	0.465	13.4
	1-Pentadecene	0.6840	0.0622	275	0.633	14.9

an average absorptivity of 15,000 liters per mole-cm. is assumed for olefin-iodine complexes, the concentration of the complex can be calculated directly from experimental absorbance data (Table I). An illustrative calculation from these data is given below for 3-methyl-*cis*-3-hexene:

$$[I_2 \times \text{olefin}] = A/\epsilon \times \text{path length} = 0.648/15{,}000 \times 1 \text{ cm.} = 4.3 \times 10^{-5} \text{ mole per liter}$$

The equilibrium constant, K, can now be calculated from the mass law equation, using the calculated concentration of the complex and the known iodine and olefin concentrations. The K value for 3-methyl-*cis*-3-hexene is found to be 0.45 liter per mole. Similar calculations for the other olefin types show that K is of the order of magnitude of 1 liter per mole.

This approximate K value of 1 liter per mole can be used to select suitable concentrations of iodine and olefins. Calculation shows that it is not possible to use an excess of iodine so as to make the absorbance independent of iodine concentration. The feasible approach is to use concentrations such that the absorbance is proportional to the product of the iodine and olefin concentrations. This will be nearly true for concentrations for which 5% or less of both components are in the complexed state. Such an approach has an important analytical advantage—the added concentrations of olefin and iodine can be used to calculate the absorptivity of the complex, since little has been used to form the complex.

The concentrations of 0.002 mole per liter of iodine and 0.03 to 0.1 mole per liter of olefin used for analysis meet the conditions discussed above. These concentrations permit the use of standard 1-cm. absorption cells. Furthermore the background absorbance due to uncomplexed iodine does not rise above 0.2 in the 275- to 400-mμ region.

The justification for the use of an "absorptivity," ϵ', (see calibration section), is that the absorbance is proportional to the product of the iodine and olefin concentrations within the selected range of concentrations. Therefore, the ϵ' can be defined as the absorbance divided by the product of iodine and olefin concentrations. This is analogous to the conventional molar absorptivity, ϵ, defined as the absorbance divided by the concentration of a single component. The apparent molecular absorptivity, ϵ', as defined in this paper is related to the true molecular absorptivity, ϵ, of the complex as follows: $\epsilon' = K\epsilon/M$ (K is the *equilibrium constant* of formation of the complex in liters per mole, and M is the molecular weight of iodine in grams per mole). Finally, the iodine concentration is expressed in grams per liter rather than in moles per liter in order to obtain an absorptivity unit of convenient numerical size.

Experiments were carried out to show that, for the conditions chosen, the absorbance measured is proportional to the product of iodine and olefin concentration. In view of the 30- to 100-fold mole ratio of olefin to iodine which is used, a very slight reaction of iodine to form diiodo compounds would apparently destroy the desired relationship. For this experiment a propylene polymer was used as the test olefin. Table II shows that the ϵ' values at 295, 317, and 337 mμ are independent of concentration even though the mole ratio of olefin to iodine was varied from 1.4:1 to 27:1.

Table II. Constancy of Absorptivity of Iodine-Olefin Complexes for Varying Concentrations of Propylene Polymer and Iodine

Concentrations, Moles/Liter		295 Mμ		317 Mμ		337 Mμ	
Olefin	Iodine	Net A	ϵ'	Net A	ϵ'	Net A	ϵ'
0.053	0.00198	0.262	9.8	0.366	13.7	0.325	12.2
0.0127	0.00909	0.275	9.4	0.402	13.7	0.354	12.0

Photochemical Effect. It is important for the present method that no appreciable fraction of the iodine added be consumed by reaction with the olefins to form diiodo compounds. The authors have found that ordinary room light (from fluorescent bulbs and north daylight) catalyzes the undesired reaction. Figures 2 and 3 show that the spectra of iodine-olefin complexes are stable if the solutions are kept in the dark, but that a change occurs if the solutions are exposed to light. The authors find that the simpler olefins react appreciably in a few minutes if exposed to light, whereas the more fully substituted olefins react much more slowly.

Another interesting proof that iodine adds to olefins in the

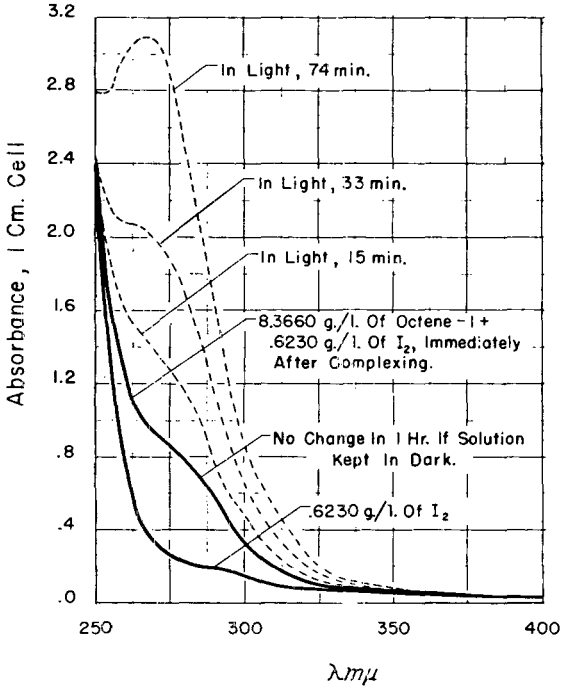

Figure 2. Effect of light on spectra of 1-octene-iodine complex

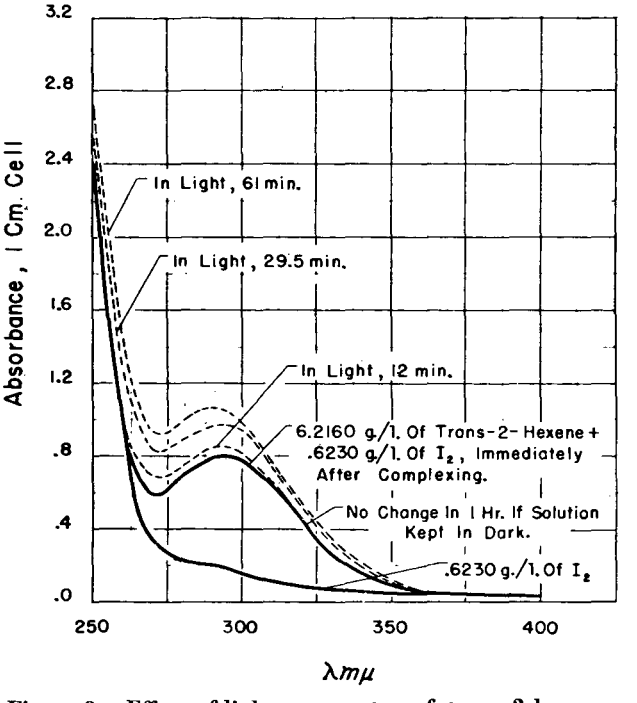

Figure 3. Effect of light on spectra of *trans*-2-hexene-iodine complex

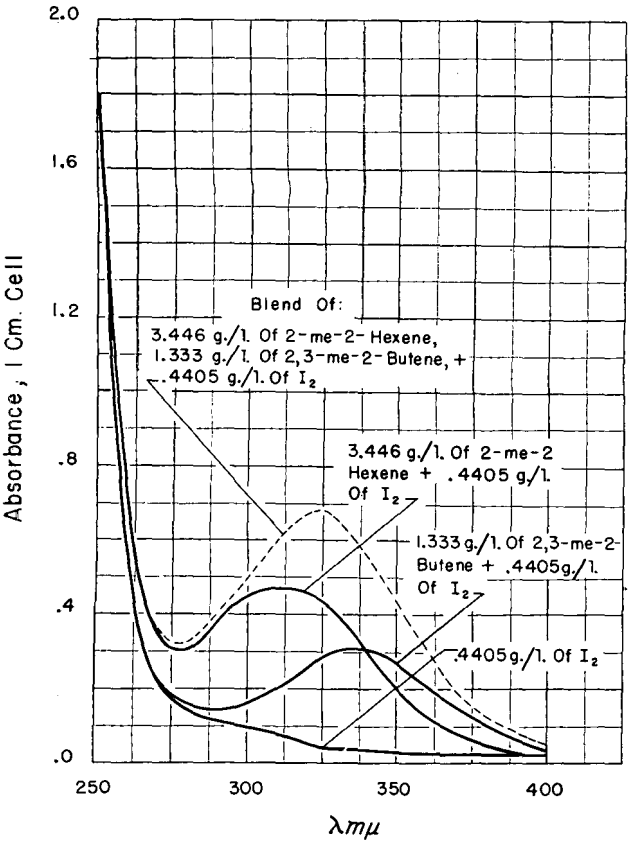

Figure 4. Analysis of two-component olefin blend by means of iodine complexes

presence of light is observable by means of the iodine absorption spectrum in the visible region. Iodine in iso-octane solution has only one band in this region, a broad one with $\lambda_{max.}$ = 520 mμ. The authors have calculated that ca. 2% of the iodine added is used to form the olefin complex under the chosen concentration conditions. This was verified by an immediate decrease of 2% in the 520-mμ band intensity when 0.07 mole per liter of *trans*-2-hexene was added to a solution of iodine in iso-octane (0.002 mole per liter). After this solution was exposed to room light for 2 hours, the 520-mμ band had decreased another 14% in intensity, indicating that addition of iodine to the double bond had occurred.

For satisfactory analytical work with olefin-iodine complexes it is therefore concluded that the spectra should be measured within a short time (ca. 1 minute) after mixing the components. Alternatively, the solutions can be preserved unchanged for at least an hour if kept in the dark until measured. It should be noted that no catalytic effects are observed from the low-intensity, monochromatic radiation incident on the sample during the absorption measurements.

Interferences. So far the method has been applied to mixtures containing only olefins, so there are not many data available on interfering substances. However, it is known that saturated hydrocarbons would not affect the analysis (*7*). Aromatic hydrocarbons will interfere because their iodine complexes overlap those of the olefins and are of comparable apparent intensity (*7*). It may be feasible to correct for the interference of particular aromatics if their concentrations are determined independently. Diolefins add iodine rapidly at room temperature (*5*), and would therefore interfere.

It has been found that the olefin analysis is not appreciably affected by the traces of color bodies and peroxide present in a propylene polymer sample that is a few days old. Although the spectral background is markedly lowered by a bulb-to-bulb vacuum distillation of such a sample, no change in the net absorbance due to the iodine complex is observed.

Correlation of Olefin-Iodine Complexes with Molecular Structure. Iodine complexes for a variety of structural types of olefins have been measured (Table I). The following conclusions from these data appear to be justified:

1. The olefins can be arranged by degree of alkyl substitution, and each type then has an approximately constant $\lambda_{max.}$ and ϵ', as follows:

Type	$\lambda_{max.}$, Mμ	ϵ'
$RCH{=}CH_2$	275	12
$R_2C{=}CH_2$	290–295	25
cis-RCH=CHR	295–300	19
trans-RCH=CHR	295–300	11
$R_2C{=}CHR$	317	27
$R_2C{=}CR_2$	337	23

More limited subclasses such as linear olefins of the $RCH{=}CH_2$ type, or α- and β-methyl substituted olefins of this class, have a considerably more constant molecular absorptivity, ϵ'. In general the absorptivities, ϵ', increase markedly with increasing alkyl substitution of the ethylenic hydrogens.

2. The trans forms of the RCH=CHR type have considerably lower values of ϵ' than the corresponding cis forms.

3. With few exceptions, branching at a carbon atom α- or β- to the ethylenic carbon atom considerably lowers the ϵ'. This effect is evident for such olefins as 4,4-dimethyl-*trans*-2-pentene and 4,4-dimethyl-1-pentene. This marked effect of branched R groups is the reason that no statement of average deviations is given for the ϵ' values in the above table. No olefins of the $R_2C{=}CHR$ and $R_2C{=}CR_2$ types with branched-chain R groups are available from API Project 44, and it would be misleading to give an average deviation figure based only on olefins with unbranched R groups.

4. Cyclopentene and cyclohexene form olefin complexes with $\lambda_{max.}$ and ϵ' values typical of cis open-chain olefins of the RCH=CHR type. However, cyclohexene has the highest ϵ' observed for any cis olefin.

APPLICATIONS TO ANALYSIS

Ordinary Quantitative Analysis. The iodine complex procedure can be used to make a quantitative determination of particular olefins which are qualitatively known to be present in a mixture. Figure 4 illustrates such an analysis for a two-component test blend containing 72.1 weight % of 2-methyl-2-hexene and 27.9 weight % of 2,3-dimethyl-2-hexene.

The characteristic wave lengths of 317 and 337 mμ are used for analysis, since these are tri- and tetrasubstituted olefins. The only unusual step is in calculating absorptivities, a', as the quotient of the absorbance and the product of olefin and iodine concentrations (see calibration). Otherwise the calculation follows the conventional spectroscopic procedure involving the usual linear simultaneous equations. The results are shown in Table III.

A more difficult analysis of the conventional type (as distinguished from a type analysis) is shown in Table IV.

Table III. Analysis of Two-Component Olefin Blend

Olefin Type	Compound	Known Value, %	Analysis by Iodine Complex, %
$R_2{=}CHR$	2-Methyl-2-hexene	72.1	71.5
$R_2C{=}CR_2$	2,3-Dimethyl-2-hexene	27.9	28.5

Table IV. Analysis of a Five-Component Olefin Blend

Olefin Type	Compound	Known Value, %	Analysis by Iodine Complex, %
$R_2C{=}CR_2$	2,3-Dimethyl-2-hexene	16.9	18.3
$R_2C{=}CHR$	3-Methyl-*cis*-3-hexene	25.4	26.1
$R_2C{=}CH_2$	2,3-Dimethyl-1-butene	19.1	17.3
cis-RCH=CHR	2,2-Dimethyl-*cis*-3-hexene	20.9	22.5
$RCH{=}CH_2$	1-Tetradecene	17.7	19.5
		100.0	103.7

Examination of a Propylene Polymer. Limited success has been achieved in extending the iodine-olefin procedure to an olefin-type analysis of a complex olefin mixture. A propylene polymer was selected because it is thought to consist largely of tri- and tetrasubstituted olefins which are detectable by the iodine-olefin procedure.

It was thought best to analyze such a complex olefin mixture by a combination infrared and iodine-olefin method. This takes advantage of the certainty with which the simpler olefin types can be determined by infrared spectroscopy. The infrared method used is essentially equivalent to that published by Saier and others (*9*), except that a 0.1-mm. cell and *n*-heptane were used.

The matrix used for the iodine-olefin procedure, including the overlaps for the olefins determined by infrared analysis, is given in Table V.

Table V. Matrix for Combined Infrared and Iodine Complex Methods for Olefin-Type Analysis

	Molecular Absorptivity, ϵ'					
$\lambda_{m\mu}$	*cis*-RHC=CHR	R_2C=CHR	R_2C=CR_2	*trans*-RHC=CHR	RCH=CH_2	R_2C=CH_2
295	18.5	17.1	8.6	11.3	5.5	24.8
317	11.6	27.0	22.3	8.6	0.9	12.7
337	3.1	19.2	33.1	2.6	0.3	3.1

Table VI. Analysis of Propylene Polymer

Olefin Type	Percentage
RCH=CH_2	2.4
R_2C=CH_2[a]	7.0
trans-RCH=CHR[a]	9.1
cis-RCH=CHR	1.6
R_2C=CHR	30.6
R_2C=CR_2	16.0
Total	66.7 (not normalized)

[a] Determined by infrared analysis.

The propylene polymer used was prepared by polymerization of *propylene* over UOP solid phosphoric acid catalyst. Its average molecular weight was approximately C_{10} *as determined by boiling* range. The analysis obtained is given in Table VI.

The low total obtained in Table VI is believed to be due to the lack of tri- and tetrasubstituted olefins with branched-chain R groups for calibration. It has been pointed out that branching of the R groups of the simpler olefin types generally decreases the absorptivity, ϵ'. It is possible that this effect is even more pronounced for the tri- and tetrasubstituted olefins. The propylene polymer is thought to contain principally these highly branched structures.

This type analysis is the first spectroscopic determination that the R_2C=CR_2 type of olefin is present in propylene polymer. Further, the R_2C=CHR type is shown to be a major constituent. This confirms infrared evidence based on a broad, general absorption in the 800- to 850- cm.$^{-1}$ region.

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Analysis of Solutions Containing Two Reducible Substances by Polarography and Coulometry at Controlled Potential

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A new analytical technique which can be applied to the determination of two substances which give overlapping polarographic waves is based on the measurement of two independent properties of the solution—the total diffusion current of the combined wave, and the total normality of reducible material in the solution. The theory of the method is outlined and the criterion for its success defined. Data secured in analyses of mixtures of thallium(I) and lead(II) are presented.

THE problem posed in polarographic analysis by the presence in a solution of two substances whose waves overlap or coincide is encountered frequently, and is one for which two methods of attack have been proposed. One of these is the Heyrovský-Bures technique (*2*), which requires the absence of any supporting electrolyte, which is based on a difference between the transference numbers of the reducible substances, and which requires no separation of the waves. The other was developed by Frisque, Meloche, and Shain (*1*); it is based on a difference between the half-wave potentials of the waves, and fails when no such difference exists (as in the case of two substances reducible at zero applied potential).

This communication describes a technique which is based on a difference between the diffusion coefficients of the reducible substances, and which is applicable even if their half-wave potentials are identical. It involves the polarographic measurement of the total diffusion current of the combined wave and the determination, by coulometry at controlled potential, of the total number of milliequivalents of reducible substance in a known volume of the solution.

This technique is not the first in which two independent properties of a solution are measured and used to establish two simultaneous equations involving the two unknown concentrations. MacNevin, Baker, and McIver (*4*) recently described a coulogravimetric procedure for the simultaneous determination of halides in their mixtures. In principle, techniques of this kind should be of great utility in the analysis of two-component mixtures. Practically, however, they all suffer from the inherent limitation that the experimental errors are multiplied many

times in the determination of a small amount of one of the substances in the presence of a large amount of the other.

THEORY

Assume that a solution contains two reducible species, A and B. At a potential on the plateau of the wave of each, the total diffusion current, i_d, is given by

$$i_d = (i_d/C)_A C_A + (i_d/C)_B C_B \quad (1)$$

where C_A and C_B are the concentrations of A and B in millimoles per liter, and the ratios $(i_d/C)_A$ and $(i_d/C)_B$ are the experimentally measured values with pure solutions of A and B separately under exactly the same conditions as those to be used in the analysis of the unknown.

Now suppose that a known volume, V ml., of the unknown solution is electrolyzed with a mercury cathode whose potential is maintained constant at a value on the plateau of the total wave. Under these conditions the two reducible substances are reduced together, and the total quantity of current consumed during an electrolysis with 100% current efficiency for the combined process is given by

$$Q = V(n_A C_A + n_B C_B) \quad (2)$$

If V is expressed in milliliters and C_A and C_B in millimoles per liter, Q is given in microfaradays. The quantities, n_A and n_B, are the numbers of faradays consumed in the reduction of 1 mole of A and B, respectively, according to the equations

$$A + n_A e = A' \quad \text{and} \quad B + n_B e = B'$$

where A' and B' may be either metal amalgams or reduction products which remain in solution.

Combining of Equations 1 and 2 gives, finally

$$C_A = [i_d - (i_d/C)_B(Q/n_B V)]/[(i_d/C)_A - (n_A/n_B)(i_d/C)_B] \quad (3)$$

and

$$C_B = [i_d - (i_d/C)_A(Q/n_A V)]/[(i_d/C)_B - (n_B/n_A)(i_d/C)_A] \quad (4)$$

In each of these equations both the numerator and denominator become equal to zero, and the entire expression consequently indeterminate, when $n_A(i_d/C)_B = n_B(i_d/C)_A$. Replacing the values of (i_d/C) with their equivalents as given by the Ilkovič equation, the criterion of failure becomes

$$n_A(607\, n_B D_B^{1/2} m^{2/3} t^{1/6}) = n_B(607\, n_A D_A^{1/2} m^{2/3} t^{1/6})$$

or, simply,

$$D_A = D_B$$

Obviously, the greater the difference between the diffusion coefficients of A and B, the greater will be the accuracy attainable.

EXPERIMENTAL

These relationships were tested by applying them to the analysis of synthetic solutions of lead and thallous ions in a 1M hydrochloric acid supporting electrolyte.

Measurements of the diffusion currents secured with various known concentrations of lead ion in this medium with 0.002% Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) gave a mean value of $(i_d/C)_{Pb}$ of 8.776 μa. per millimole per liter. The corresponding value for thallous ion was found to be 5.975. According to Lingane (*3*), the diffusion current constant of lead ion in 1M hydrochloric acid is 3.86, whereas earlier work in this laboratory (*6*) gave the diffusion current constant of thallous ion in 1M hydrochloric acid as 2.731. The ratio (I_{Tl}/I_{Pb}) calculated from these data, 0.6816, is in good agreement with the present value of the equivalent ratio $(i_d/C)_{Tl}/(i_d/C)_{Pb} = 0.6808$. Assuming the correctness of the diffusion coefficient term in the Ilkovič equation, it follows that in 1M hydrochloric acid $D_{Tl}/D_{Pb} = (4)(0.68)^2 = 1.85$.

Known volumes of standard lead and thallous solutions were mixed in a calibrated volumetric flask and diluted to volume with 1M hydrochloric acid. A 9.995-ml. aliquot of this solution was diluted to 100.0 ml. with 1M hydrochloric acid containing 0.002% Triton X-100, and the diffusion current was measured at −0.70 volt *vs.* S.C.E. after thorough deaeration. This value was multiplied by 100.0/9.995 to secure the diffusion current which would have been measured in the original undiluted solution.

Meanwhile about 100 ml. of 1M hydrochloric acid was electrolyzed in a diaphragm cell (*5*) with a mercury cathode whose potential was maintained constant at −0.70 volt *vs.* S.C.E. by an Analytical Instruments, Inc., (Bristol, Conn.) potentiostat. A rapid stream of nitrogen was bubbled through the solution in the cathode compartment continuously to keep it free from oxygen, which is reducible at this potential. A platinum wire anode was used, and a small amount of hydrazine hydrochloride was added to the acid in the anode compartment to prevent attack on the platinum.

When the current during this pre-electrolysis had fallen to a negligibly small value, the register of an Analytical Instruments, Inc., current integrator (*5*) in series with the cell was read. The electrolysis circuit was then disconnected and exactly 20.003 ml. of the unknown solution was pipetted into the cathode compartment. A minute or two was allowed for removal of dissolved oxygen by the stream of nitrogen; then the electrolysis circuit was reconnected, and the electrolysis was allowed to proceed unattended for about 1 hour. At the end of this time the current had fallen to 0.01% or less of its initial value. The integrator was read again: with the 10-ohm input resistor used throughout this work, one count corresponded to 0.099995 microfaraday, so that the value of Q required in the calculations was secured simply by dividing the number of counts accumulated during the electrolysis by 10. Further details concerning the performance of precise controlled potential coulometric analysis are given in an earlier paper (*5*).

The data shown in Table I were secured in the analysis of a solution made up to contain 1.684mM lead and 4.012mM thallous ions, and the values found were in error by about ±0.7%. In a series of five independent analyses of this solution the mean error was about ±1.3%, as shown in Table II.

Table I. Analysis of Typical Thallium-Lead Solution

$[C_{Pb} = 1.684\text{m}M;\ C_{Tl} = 4.012\text{m}M]$

$n_{Tl} = 1$; $(i_d/C)_{Tl} = 5.975$ μa. per millimole per liter

$n_{Pb} = 2$; $(i_d/C)_{Pb} = 8.776$ μa. per millimole per liter

$i_d = (3.876)(100.0)/9.995 = 38.77_9$ μa.

$Q/V = 147.58/20.003 = 7.377_9$ microfaraday/ml.

$$C_{Pb} = \frac{38.779 - (5.975)(7.3779)}{8.776 - (2)(5.975)} = \frac{38.779 - 44.083}{-3.174} = 1.671\text{m}M$$

$$C_{Tl} = \frac{38.779 - (1/2)(8.776)(7.3779)}{5.975 - (1/2)(8.776)} = \frac{38.779 - 32.374}{1.587} = 4.036\text{m}M$$

$\Delta C_{Pb} = -0.7_7\%$; $\Delta C_{Tl} = +0.6_0\%$

$Q_{theor.} = 147.62$ microfaraday; $\Delta Q = -0.02_7\%$

$i_{dtheor.} = 38.751$ μa.; $\Delta i_d = +0.07_2\%$

Table II. Analyses of Thallium-Lead Solutions

$[Tl^+]$, mM			$[Pb^{++}]$, mM			
Taken	Found	Error, %	Taken	Found	Error, %	$[Tl^+]/[Pb^{++}]$
4.012	4.122	+2.7	2.446	2.393	− 2.1	1.6
4.012	4.036	+0.6	1.684	1.671	− 0.8	2.4
	3.935	−1.9		1.725	+ 2.4	
	4.050	+0.9		1.662	− 1.3	
	4.101	+2.2		1.640	− 2.6	
	4.043	+0.8		1.669	− 0.9	
6.398	6.400	±0.0	1.684	1.683	− 0.1	3.8
	6.325	−1.1		1.701	+ 1.0	
6.398	6.439	+0.6	1.221	1.200	− 1.7	5.2
	6.207	−3.0		1.288	+ 5.5	
4.012	4.063	+1.3	0.245	0.219	−10.6	16
	4.056	+1.1		0.222	− 9.4	
6.398	6.416	+0.3	0.122	0.102	−16.4	52

The propagation of errors in this experiment is illustrated by the last two lines of Table I, which give the differences between the measured values of i_d and Q and the corresponding values computed from the known thallium and lead concentrations. In this instance the experimental errors are magnified roughly tenfold in the concentrations computed from them. As Table II shows, good results were secured in analyses of solutions in which the thallium to lead ratio was as high as 50.

This magnification of experimental errors will become more serious as the diffusion coefficient ratio decreases below its value

in this instance of 1.85. Results of some value, however, should still be available with a ratio of diffusion coefficients as low as about 1.4, but this should probably be considered the lower limit of feasibility of the method until further refinements of experimental technique have materially reduced the errors of measurement.

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Cells, Apparatus, and Methodology for Precise Analysis by Coulometry at Controlled Potential

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The failure of controlled potential coulometric analysis to be used in the analytical laboratory has been due primarily to the lack of an accurate and precise direct-reading current integrator. The construction and operation of such an instrument are discussed, together with two cells suitable for rapid controlled potential electrolyses, and the general methodology of precise analysis by coulometry at controlled potential is outlined. Determinations of cupric copper, in amounts varying from 2 to 600 mg., were accurate and precise to within ±0.1%. An extrapolation technique is described which decreases the electrolysis time required to only 20 to 30 minutes.

IN principle, coulometry at controlled potential with a mercury cathode, as developed by Hickling (*3*) and Lingane (*11*), among others, has numerous advantages over ordinary electrogravimetric procedures, but in the past it has suffered from one crippling disadvantage. The advantages—the ease with which so many separations can be accomplished, the fact that a potentiostat relieves the operator for other duties throughout the whole duration of the electrolysis, and the elimination of all the operations involved in weighing a metal deposit (to say nothing of the fact that coulometric procedures need not involve the deposition of a solid metal)—have been expounded in a recent monograph by Lingane (*4*).

The crippling disadvantage has been the lack of a precise, accurate, and simple instrument for integrating the electrolysis current. The silver coulometer employed by Szebellédy and Somogyi (*16*) merely substitutes the operations of washing, drying, and weighing the silver deposited in the coulometer for the very same operations on the metal deposited in the electrolysis cell. In using Lingane's gas coulometer (*11*), liquid levels must be adjusted before, during, and after the electrolysis, the volume, temperature, and pressure of the gas evolved must be measured, and finally the volume of gas must be corrected to standard conditions. Moreover, it is now known that the hydrogen-oxygen coulometer gives seriously erroneous results with low currents (*15*).

The seeming complexity of the coulometric measurements, coupled with the fact that the precision of the available data has been "not too impressive" (*10*), has greatly retarded the adoption of controlled potential coulometric analysis as a standard technique of chemical analysis. Lingane wrote (*9*) that "the development of a direct-reading instrument, capable of integrating current-time curves with a precision and accuracy of the order of 0.1%, would be a boon to controlled potential coulometric analysis." This paper describes an instrument which fulfills these specifications, together with some details of the manner in which it may be used in practical analysis.

APPARATUS

The potentiostat and integrator used in this work were designed in collaboration with Julian M. Sturtevant. The operating characteristics of the potentiostat are as follows: range of direct current output *voltages* available, 0 to 10 and 0 to 28 volts on the low and high ranges, respectively; direct current electrolysis current available, 10 amperes at 28 volts; alternating current ripple component of direct current output, less than 0.5% (peak-to-peak) of direct current voltage per ampere; range of direct current control *potentials* available, ±3 volts; sensitivity of potential control, better than ±5 mv. under all conditions thus far investigated. Although these characteristics are generally superior to those of most previously described instruments, they have (with the exception of the attainable voltage and current) relatively little influence on its use in coulometric procedures. A description of this potentiostat, which is to be manufactured by Analytical Instruments, Inc., Bristol, Conn., will be published elsewhere.

A schematic diagram of the circuit of the integrator is shown in Figure 1. The electrolysis current is passed through one of the three standard resistors connected to the input selector switch, and the voltage developed is added algebraically to the opposing output of a direct current tachometer generator. The resulting voltage is converted to 60-cycle alternating current, is amplified, and is used to drive a two-phase servo motor. This turns the generator at a speed which produces a generator output practically equal to the iR drop through the input resistor. A five-dial revolution counter attached to the gear train is turned simultaneously at a rate proportional to the rate of revolution of the generator shaft; thus the counting rate at any instant is proportional to the electrolysis current. Accordingly, the total number of counts recorded during an electrolysis is proportional to the quantity of electricity used. This arrangement is very similar to that of the voltage integrator described by Buzzell and Sturtevant (*1*).

The three input resistors are provided to permit selection of an over-all sensitivity which gives a suitably large number of counts for any coulometric analysis. With the particular motor, generator, and gear train used, the maximum rate of revolution of the generator shaft corresponds to an output of about 1 volt. Accordingly, the 1-ohm input resistor is used in electrolyses in which the initial current may be as large as 1 ampere. If the initial current exceeds this value, the instrument cannot provide an accurate integral unless the 0.1-ohm resistor is substituted; in this way the range of the instrument is extended to maximum currents of 10 amperes, which is ample for any practical use. If, however, the initial current is less than 0.1 ampere, the use of the 10-ohm resistor is recommended to increase the number of counts recorded, and also to reduce to a minimum the unavoidable effects of friction at very low counting rates.

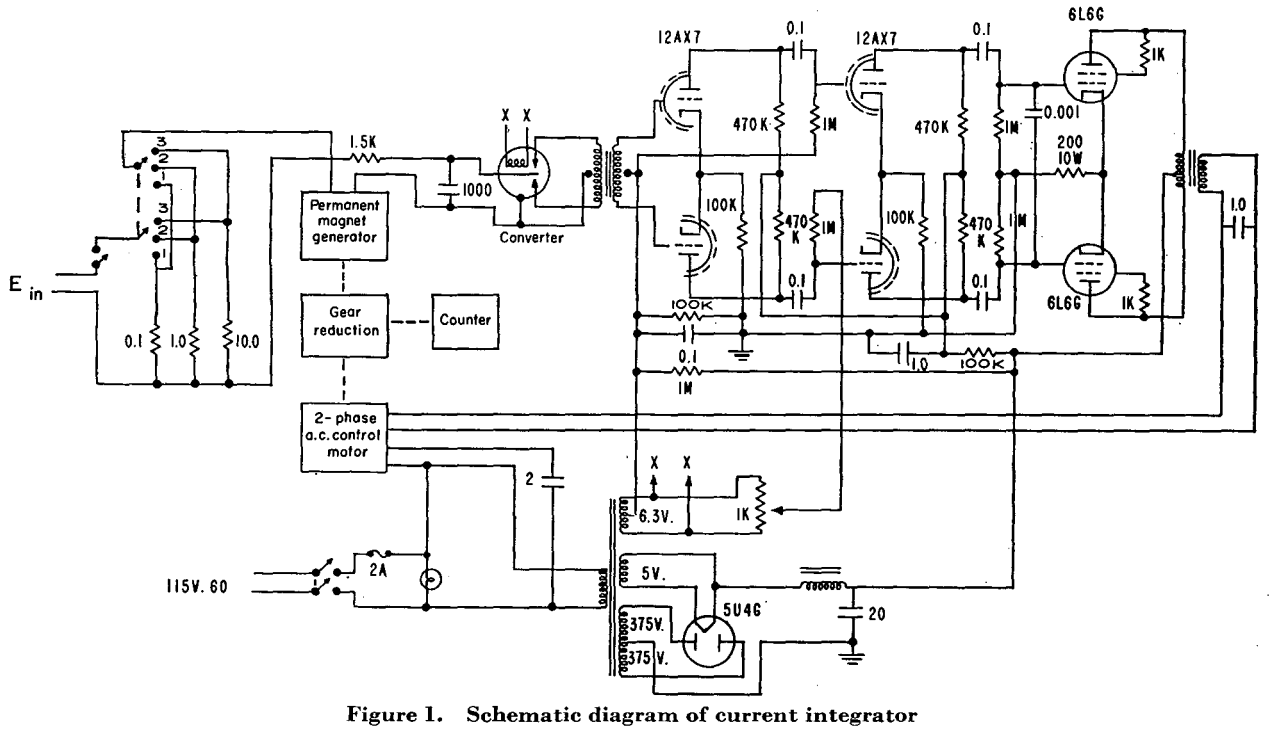

Figure 1. Schematic diagram of current integrator

Converter. Brown converter, Brown Instruments Division, Minneapolis-Honeywell Co., Philadelphia, Pa.
Input transformer. Type 1-67B, Palmer Electric and Manufacturing Co., Wakefield, Mass.
Output transformer. Type CG-16, United Transformer Corp., New York
Power supply choke. 15 millihenries, 10 amperes
Power transformer. Stancor PC-8411, Chicago Standard Transformer Corp.
Servo motor. Type FPE 25-11, Diehl Manufacturing Co., Finderne, N. J.
Generator. Type 363, Electric Indicator Co., Springdale, Conn.
Rated output. 420 mv. per 100 r.p.m.
Gear ratios. Motor to generator, 12 to 1; motor to counter, 48 to 1

A typical calibration curve is shown in Figure 2. This was secured by applying various constant voltages to a circuit comprising a precision resistor in series with the 10-ohm input resistor of the integrator, and measuring the iR drop across the external resistor with a carefully calibrated Rubicon potentiometer. The quantity of electricity flowing in each calibration was computed from the known steady current and the measured duration of the experiment.

Table I. Effect of Input Resistor on Sensitivity of Current Integrator

Input Resistor, Ohms	Sensitivity, Microfaradays per Count[a]		
	Nominal	Observed	Difference, %
1	1	1.00082	$+0.08_2$
10	0.1	0.099995	-0.00_5^5
100	0.01	0.0100035	$+0.03_5^5$
1000	0.001	0.00099976	-0.02_4
10000	0.0001	0.000099195	-0.80_5

[a] 1 microfaraday = 0.096493 coulomb.

These data show that with the 10-ohm input resistor in series with the electrolysis cell, the sensitivity of the integrator is constant and equal to 0.099995 microfaraday per count for input currents between 1.5 and 105 ma. Table I shows that, with the exception of the data secured with an input resistor of 10,000 ohms (which would be used only for integrating currents smaller than 100 μa.) the sensitivity is changed by a factor of almost exactly 10 whenever the value of the input resistor is changed by a factor of 10. Consequently, the instrument on every range is direct reading in an appropriate decimal multiple of 1 microfaraday per count; it reads directly in milliequivalents after proper location of the decimal point. This is a very considerable advantage in routine analytical work.

The effect of the decrease of sensitivity at very low currents, which occurs with every input resistor when the iR drop across it falls below about 15 mv., is sufficiently small to cause the integrated current to be in error by less than 0.2%, if the initial current is at least one tenth of the rated maximum for the input resistor used.

The stock solution of copper(II) sulfate had been standardized

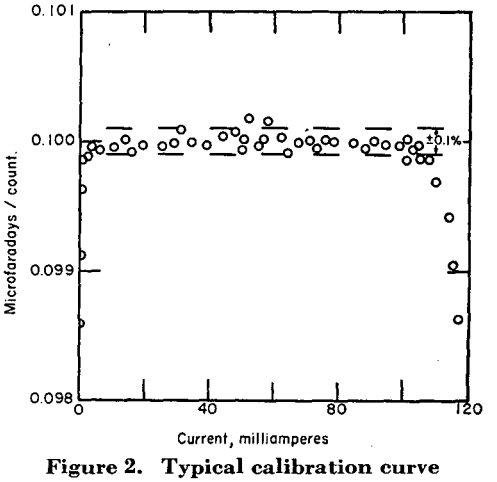

Figure 2. Typical calibration curve

Secured with 10-ohm input resistor

repeatedly by a very precise iodometric method (*14*), and had a known normality of better than ±0.02%. Aliquots of this solution, taken with carefully calibrated pipets, were used to provide known amounts of copper. All other chemicals were ordinary reagent grade.

CELLS

According to the equation deduced by Lingane (*5*) for the current-time curve during a controlled potential electrolysis, the rate of such an electrolysis is directly proportional to the ratio of cathode area to solution volume. In a cell of cylindrical cross section, Lingane found (*4*) that the value of k in the equation

$$i_t = i_o 10^{-kt}$$

was generally about 0.037. Thus, 54 minutes are required to drive a reaction 99% of the way to completion in such a cell,

or 81 minutes to achieve 99.9% completion, and very much longer to secure the maximum possible degree of separation (*8*). This is a considerable drawback to the use of coulometry at controlled potential in the routine laboratory. In the cells of Figures 3 and 4, the area to volume ratio is increased by using a conical working electrode compartment, with the result that 99% completion can be attained within 24 minutes, and 99.9% completion within 35 minutes.

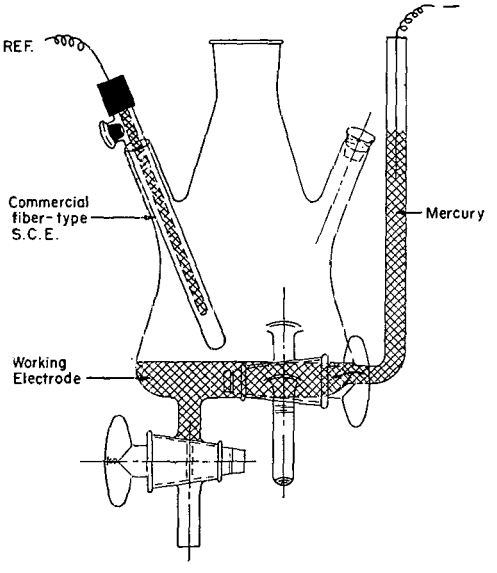

Figure 3. Cell for controlled potential electrolysis with mercury cathode and internal auxiliary electrode

The cell shown in Figure 3 was designed for electrolytic separations with a mercury cathode at controlled potential and an internal auxiliary electrode, and may be used for coulometric analyses when a suitable anodic depolarizer can be found. It consists of a three-necked 125- or 250-ml. Erlenmeyer flask with a side tube carrying a stout piece of platinum wire and filled with mercury for electrical connection to the potentiostat. The central neck of the flask carries a one-hole rubber stopper through which the stirrer shaft passes. Around this shaft is wound a helix of platinum or silver wire which serves as the auxiliary electrode and is connected to the potentiostat by means of an alligator clip just above the stopper. One of the off-center necks of the cell carries a commercial 5-inch fiber-type saturated calomel electrode whose tip just trails in the mercury; the other neck is used for the addition of reagents during the electrolysis. Stopcocks are provided for emptying the cell completely and for removing a portion of the solution without interrupting the electrolysis.

The more elaborate cell shown in Figure 4 is necessary for analyzing solutions—e.g., of copper(II) in ammoniacal medium—for which a suitable auxiliary electrode-depolarizer combination is impossible to find. Diaphragm cells for coulometry at controlled potential have been described by Lingane, Swain, and Fields (*12*) and by Diehl (*2*). However, neither of these is wholly practical (*7*), for it is not simple to prevent bulk flow of the solution between the two compartments without unduly increasing the cell resistance. These difficulties are surmounted in the cell of Figure 4 by the use of a double diaphragm enclosing a central compartment filled with the supporting electrolyte present in the working electrode compartment. The latter is separated from the central compartment by a 20-mm. fine-porosity sintered disk, and the central compartment is separated from the auxiliary electrode compartment by a medium-porosity disk of equal size. The second disk does not appreciably increase the total cell resistance (which is usually of the order of 10 ohms), and serves only to prevent convective mixing of the solutions in the central and auxiliary electrode compartments. Stoppering the central compartment reduces the rate of equalization of pressure across the diaphragms, and permits reducing the rate of flow of liquid into the working electrode compartment to 1 ml. per hour or less. Even this negligible volume of solution is practically free from the products of the reaction at the auxiliary electrode.

Since not every potentiostat will tolerate the resistance (about 2500 ohms) of a commercial fiber-type calomel electrode in its control circuit (*6*), it may be desirable to use some other type of reference electrode when working with another potentiostat.

These cells are available commercially from Analytical Instruments, Inc., Bristol, Conn.

EXPERIMENTAL

All except a few preliminary experiments were made with the double-diaphragm cell described, employing the following procedure. The entire cell was emptied and the central compartment was filled with the supporting electrolyte and was stoppered. More of the same solution was added to the working electrode compartment to cover the gas dispersion cylinder, through which a rapid stream of nitrogen or hydrogen was bubbled. The auxiliary electrode compartment finally was filled with the same solution to a point about 3 cm. higher than the level in the working electrode compartment. The stirrer was started, and 30 ml. of mercury was added to the working electrode compartment with the potentiostat adjusted to maintain the cathode potential constant at the desired value. A stirrer speed should be selected so that the surface of the mercury is in as rapid motion as possible without causing the formation of detached droplets of mercury. This stirrer speed, by minimizing the thickness of the diffusion layer, serves to increase the currents secured and thus the rate at which the electrolysis proceeds.

Pre-electrolyzing the supporting electrolyte alone for a few minutes served to remove not only residual traces of oxygen but also traces of reducible material contained in the chemicals from which the supporting electrolyte was composited. The course of this pre-electrolysis was followed by using the integrator with an input resistor ten times as large as that to be used in the subsequent analysis. When the counting rate under these conditions had dropped to 0.1 count per minute or less (0.01 count per minute in the actual analysis), the electrolysis circuit was disconnected (a switch for this purpose is provided on the Analytical Instruments, Inc., potentiostat) and the desired volume of cupric solution was added to the cell. When, after a minute or two, during which the appropriate input resistor of the integrator was connected into the circuit, the oxygen contained in the sample solution had been removed by the nitrogen stream, the integrator register was read to the nearest 0.1 count, the electrolysis circuit was reconnected, and the electrolysis was allowed to proceed without further attention.

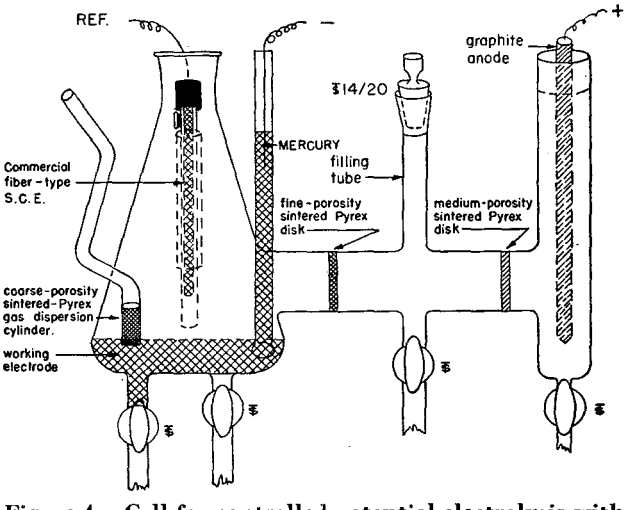

Figure 4. Cell for controlled potential electrolysis with mercury cathode and external auxiliary electrode

When the total volume of solution in the cathode compartment was just sufficient to cover the sintered disk separating it from the central compartment, the rate at which the electrolysis proceeded was such that 99.99% completion was reached in about 45 to 50 minutes. After this length of time, the register was read again. Subtraction of the initial from the final register reading, followed by appropriate location of the decimal point, gave the number of milliequivalents of copper directly. Some data secured by this procedure in several different supporting electrolytes are shown in Table II. The accuracy and precision of these data compare very favorably with those obtained in electrogravimetric work. Although each analysis consumed about 60 to 75 minutes, the operator's attention was required during only a small fraction

Table II. Coulometric Determination of Copper

(Volume of solution was generally 100 to 150 ml., and of mercury cathode was 30 to 35 ml.)

Supporting Electrolyte, 1 *M*	E_c *vs.* S.C.E., Volts	*n*	Meq. Taken	Meq. Found	Error, %
H_2SO_4	−0.50	2	9.476	9.503	+0.29
			9.476	9.451	−0.26
			7.105	7.103	−0.02
			4.736	4.733	−0.07
			4.736	4.737	+0.03
			2.349	2.352	+0.16
			1.4183	1.4178	−0.04
			0.7070	0.7073	+0.04
			0.4672	0.4668	−0.09
			0.2373	0.2375	+0.07
			0.07546	0.07534	−0.15
HCl	−0.10	1	4.738	4.734	−0.08
			1.1745	1.1748	+0.03
	−0.50	2	9.476	9.488	+0.13
			2.349	2.348	−0.04
NH_3–NH_4Cl	−0.75	2	7.105	7.089	−0.10
			0.7070	0.7068	−0.03
$HClO_4$	−0.50	2	18.96	18.96	±0
			2.349	2.346	−0.13
				Mean	−0.01 ± 0.09

Table III. Extrapolation to Zero Current

(Electrolysis of 6.482 meq. of Cu^{++} in 150 ml. of 1*M* HCl E = 0.45 volt *vs.* S.C.E.; V_{Hg} = 35 ml.)

Elapsed Time, Min.	Register Reading (1 Count = 0.001 Meq.)	Counts per Minute		Extrapolated Reading
0	29230.2			
20	35364	165	46	
		88.0	22	
25	35529		24	35529 + (24/22)(165) = 35709.0
		45.5	11.6	
30	35617.0		12.4	35617.0 + (12.4/11.6) (88.0) = 35711.1
		22.8	6.0	
35	35662.0		6.4	35662.0 + (6.4/6.0)(45.5) = 35710.5
		11.5	2.9	
40	35684.8		3.3	35684.8 + (3.3/2.9)(22.8) = 35710.7
			1.6	
45	35698.3		1.7	35698.3 + (1.7/1.6)(11.5) = 35710.5
		Mean extrapolated value		35710.4 ± 0.5
		Expected final reading		35712
		Error, %		−0.025

of this time, and washing, drying, and weighing of a solid electrode were entirely eliminated.

Moreover, the total time required could be decreased considerably by employing an extrapolation method for estimating the last few per cent of the quantity of electricity consumed in an electrolysis. This technique, whose theoretical foundation is identical with that of the MacNevin and Baker procedure (*13*), is illustrated by the data in Table III, which were secured during the electrolysis of a 1*M* hydrochloric acid solution containing 6.482 meq. of cupric copper. With the 1-ohm input resistor used, a total of 6482 counts should have been recorded. Nineteen and a half minutes after the beginning of the electrolysis—the time was chosen solely for symmetry in constructing the table, and, as is not true of the MacNevin and Baker technique, is of no importance in the calculations—when about 5% of the original amount of copper remained undeposited, the register was read and again was read exactly 1 minute later. During this 1-minute period 46 counts were recorded. This is practically equal to the instantaneous counting rate at a register reading equal to the mean of the values at 19.5 and 20.5 minutes, or 35364. The measurements were repeated at 24.5 and 25.5 minutes, and the counting rate then was found to be 24 counts per minute at a register reading of 35529. Consequently 35529 minus 35364 (=165) counts were required to decrease the counting rate (which is proportional to the electrolysis current) from 46 to 24 counts per minute. To decrease the counting rate to zero, an additional (24/22) × 165 (=180) counts would be required. From this the final register reading would be expected to be 35529 + 180 (=35709), which corresponds to a total of 6479 counts and to an error of only −0.05%. The remaining data were treated in exactly the same way, with the results shown in the last column of Table III. Results of this technique are accurate to within a few counts with a saving up to one half of the total time required for an analysis, and a controlled potential coulometric analysis may be carried out within about 0.5 hour and with an average precision of ±0.1%.

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High Frequency Combustion-Volumetric Determination of Carbon in Metals

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An examination has been made of the variables present in the high frequency combustion-volumetric method for the determination of carbon in metals, and methods of controlling or correcting for them are discussed. The experimental work was done with the commercially available Lindberg equipment. The standard deviation for a single determination has been established as one division of the Lindberg gas buret scale, which corresponds to 0.005% carbon for a 1-gram sample when the gas is measured at 20° C. and 760 mm. of mercury.

THE convenience of high frequency induction heating devices for the determination of carbon in metals arises from the fact that they permit rapid combustion of the sample in a total volume of oxygen (about 500 cc.) which is small in comparison with the volumes used in more conventional combustion equipment. It therefore becomes possible to substitute a rapid volumetric analysis of the combustion products for the gravimetric determination of carbon dioxide generally employed.

A previous publication (*6*) described a precision determination of carbon in metals using a modified Lindberg high frequency

combustion furnace and volumetric carbon determinator (*5*). Replicate analyses on seven standard steels were reported with a mean error of 0.004% carbon and a lower limit of precise measurement of 0.02% carbon with a 1-gram sample. [The standard deviation estimated from the data is 0.006% carbon (*1*).] During continued use of similar Lindberg equipment in other laboratories of this company errors were frequently observed which were larger than those expected on the basis of the original work. This led to a re-examination of the method, and after careful consideration had been given to the measurement of and correction for certain variables in the process it became possible to operate the equipment consistently with a standard deviation of 0.005% carbon.

Accurate volumetric gas analysis requires adequate control over the variables of temperature and pressure, or application of corrections where control is not feasible. What constitutes "adequate control" will be determined both by the accuracy desired and by the volume to be measured.

In the Lindberg volumetric carbon determinator the amount of carbon dioxide in the combustion products is calculated from the difference between two gas volumes (both about 500 cc.), one measured before and the other after absorption of the carbon dioxide by a potassium hydroxide solution. As will be shown in subsequent calculations, 0.005% carbon, the desired standard deviation, is equivalent to 0.10 cc. of gas. The experimental problem, therefore, is that of measuring the difference between two gas volumes of about 500 cc. with a standard deviation of 1 part in 5000. Translating this into its equivalent in temperature and pressure control, a change of 0.10 cc. in the gas volume can be produced by a variation of only 0.05° K. between initial and final buret readings in the neighborhood of 300° K., or by a pressure variation of slightly more than 0.1 mm. of mercury in the neighborhood of 1 atmosphere. Such variations have been observed during the operation of the equipment in this laboratory.

In a conventional Orsat gas analysis apparatus the total volume measured is 100 cc. or less, so that a proportionally larger relative error in the measurement can be tolerated. Furthermore, the geometry of the Orsat buret permits more effective thermostating of the gas by the water jacket than is possible with the Lindberg buret. Finally, for precision gas analysis the Orsat buret is readily fitted with a compensator to eliminate the effects of barometric and temperature fluctuations.

VARIABLES IN GAS VOLUME MEASUREMENT

The variables involved in the operation of the Lindberg volumetric carbon determinator may be discussed in terms of Figures 1 and 2.

Temperature. The thermometer originally mounted in the buret has been replaced by one graduated in 0.1° K. increments, *H*. It is read immediately after each measurement of gas volume.

Pressure. Three factors can produce a pressure variation.

Barometric Fluctuations. The barometer is read after each measurement of gas volume if there is any indication of rapid changes in this variable.

Temperature Fluctuations. A temperature change of 0.1° K. will change the vapor pressure of the leveling fluid by slightly more than 0.1 mm. of mercury.

Lack of Internal Pressure Equilibrium. This is the major source of pressure variation, and, fortunately, is the one source which can be eliminated.

The gas is returned from the potassium hydroxide absorption vessel to the buret by applying pressure to the atmospheric side of the potassium hydroxide vessel through the opening, *K* (*6*). The inertial effect of this gas transfer is to produce, at the moment of seating the potassium hydroxide check valve, *I* (or at the moment of bringing the potassium hydroxide to a constant level mark below the check valve), a pressure gradient in the system. If the stopcock, *C*, is closed immediately upon seating the check valve, the pressure of the gas thus isolated in the buret will be slightly greater than that in the manifold between valve and stopcock.

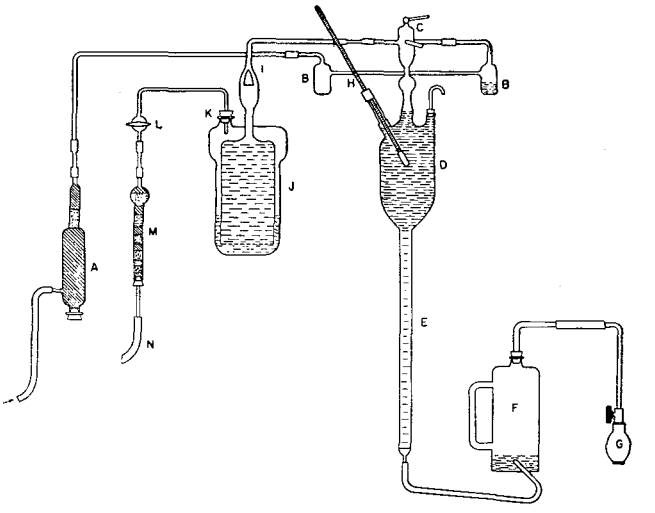

Figure 1. Diagram of modified Lindberg volumetric carbon determinator

A. Dust filter and sulfur dioxide absorbent
B. Bubbler
C. Three-way buret stopcock
D. Gas buret
E. Graduated portion of gas buret
F. Leveling bulb
G. Hand bulb
H. Thermometer, 0.1° C. graduations
I. Check valve
J. Potassium hydroxide absorption pipet
K. Opening of potassium hydroxide absorption pipet
L. Stopcock
M. Tube filled with alternate layers of Ascarite and indicating Drierite
N. Tube through which pressure can be applied to seat the potassium hydroxide pipet check valve

This effect may be eliminated by adding to the volumetric system a stopcock, *L*, at the atmospheric side of the potassium hydroxide vessel. After the gas has been transferred to the buret and the check valve seated, stopcock *L* is closed and stopcock *C* kept open. This will maintain the check valve in position and also permit internal pressure equilibrium to be established between the manifold and buret. With stopcock *L* closed the attainment of equilibrium may be facilitated by slowly raising and lowering the leveling bulb a few times until a constant buret level is attained. After equilibration, stopcock *C* is closed and the volume in the buret measured.

After each determination the manifold is brought back to atmospheric pressure by appropriate manipulation of stopcock *C* while stopcock *L* remains closed.

Water Vapor Equilibration. The vapor pressure of the potassium hydroxide absorption solution is less than that of the buret leveling fluid by about 4 mm. of mercury (*2*). If the buret has been thoroughly dri-filmed (General Electric silicone product, Dri-film 9987) a prohibitively long time is required for the attainment of water vapor equilibrium after the gas is returned to the buret from the potassium hydroxide absorption vessel (*6*). Calculations recently published by Stein and Reid (*7*) show that the gas in a buret whose sides are not wetted by the leveling fluid does not become saturated with water vapor even after 4 hours, whereas 2 to 3 seconds suffice if the buret walls are covered by a film of liquid. If the buret has been scrupulously cleaned, smooth drainage and reproducible buret readings can be achieved, and the buret can be kept in this condition if the liquid level is always kept just below the buret stopcock, *C*, when the unit is not in use. The leveling bulb itself should also be kept covered to prevent dirt contamination. With these precautions it has been possible to maintain, without further cleaning, excellent drainage characteristics in the present buret, which was thoroughly cleaned in December 1951.

Drainage. With a clean buret reproducible readings can be obtained with a drainage period of 30 seconds.

Gas Solubility. The leveling fluid used (acidified 20% aqueous sodium sulfate) is one in which oxygen and carbon dioxide have

very low solubilities. No difference in readings of gas volumes was noted between runs using fresh leveling solution and solution through which oxygen had been bubbled for 1 hour.

CORRECTION FOR VARIATIONS IN GAS TEMPERATURE AND BAROMETRIC PRESSURE DURING A RUN

The correction of a measured gas volume for changes in temperature or pressure is ordinarily a simple and straightforward gas law calculation. In the Lindberg volumetric carbon determinator, however, only part of the buret is graduated, and that part in an arbitrary scale showing per cent carbon. Thus, no direct measurement is made of either the final or initial volumes of gas. Before the gas law corrections of Equations 3 and 4 below can be applied, additional data and calculation are necessary.

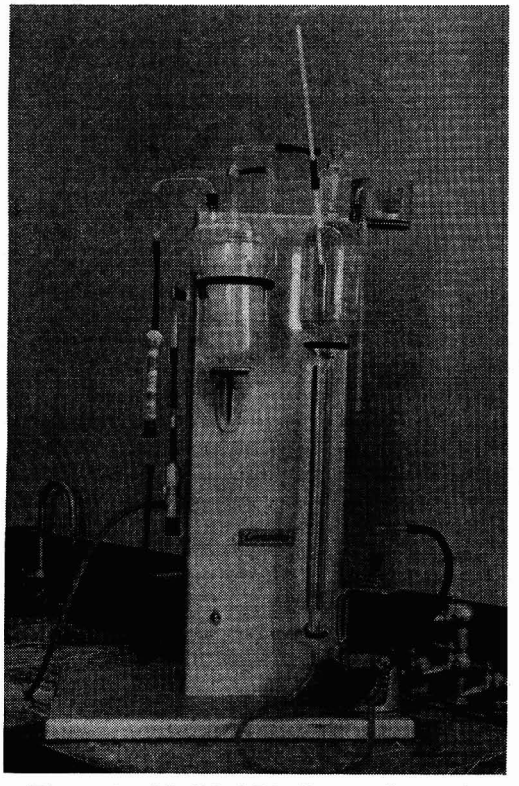

Figure 2. Modified Lindberg volumetric carbon determinator

If there were no variation in gas temperature or barometric pressure during a run the decrease in gas volume following the absorption of carbon dioxide from the carbon dioxide–oxygen mixture would be directly proportional to the number of moles of carbon dioxide as shown in Equation 1.

$$\Delta V = (V_i - V_f) = n_{CO_2}\left(\frac{RT}{p}\right) \tag{1}$$

$$p = P - p_s \tag{2}$$

in which

V_i = initial gas volume = $(n_{CO_2} + n_{O_2})\frac{RT}{p}$
V_f = final gas volume = $n_{O_2}\left(\frac{RT}{p}\right)$
P = barometric pressure (corrected to 0° C.)
p_s = vapor pressure of leveling solution
n_{CO_2} = number of moles of carbon dioxide
n_{O_2} = number of moles of oxygen

If, however, the final temperature and pressure differ from those at which the gas was collected, the observed ΔV is not proportional to n_{CO_2}. It must be corrected as follows:

$$\Delta V_{corr.} = \Delta V_{obs.} + \Delta V_f \tag{3}$$

in which ΔV_f is the change in V_f which would result upon changing gas temperature and pressure back to their original values:

$$\Delta V_f = (V_f)_{obs.} - (V_f)_{obs.}\left(\frac{p_f}{p_i}\right)\left(\frac{T_i}{T_f}\right) \tag{4}$$

In order to apply these corrections the following data are needed:

1. p_s—vapor pressure of leveling solution at any temperature. This may be estimated with sufficient accuracy for these calculations by interpolation of data available in the literature on the vapor pressures of water (*3*) and of sodium sulfate solutions and sulfuric acid solutions (*2*).

2. Relationship between ΔV as read from the buret in units of per cent carbon and ΔV in units of cubic centimeters.

When the carbon dioxide obtained by burning 1 gram of a 1% carbon sample is measured at a barometric pressure of 760 mm. of mercury (0° C.) and a temperature of 20° C. the $(\Delta V)_{\%} = 1.000$.

$\Delta V_{cc.}$ for the above conditions can be obtained from Equation 1; it is 20.46 cc.

$$20.46 \text{ cc.} = 1.000\%$$
$$1.00 \text{ cc.} = 0.049\%$$

3. Values for V_i and V_f.

The volume of the buret from the 0.000% mark to stopcock C is 550 cc., as measured by the volume of solution it will hold.

$$V \text{ (cc.)} = 550 - (20.46)(\text{buret reading}) \tag{5}$$

Illustrative Example

	Initial	Final
Buret reading, %	0.010	0.145
Gas volume, cc.		547.03 (equation 5)
Temperature, ° C.	27.30	27.20
Barometric pressure, mm. (0° C.)	742.57	742.57
p_s, mm. (estimated)	25.57	25.42
Weight of sample	1.063 grams of NBS 15e (0.107% C)	

From Equation 4

$$\Delta V_f = 547.03 - 547.03\left(\frac{742.57 - 25.42}{742.57 - 25.57}\right)\left(\frac{273.2 + 27.3}{273.2 + 27.2}\right)$$
$$= -0.27 \text{ cc.}$$
$$(\Delta V_f)_{\%} = (-0.27 \text{ cc.})(0.049\%/\text{cc.}) = -0.013\%$$
$$(\Delta V)_{\%(corr.)} = (0.145 - 0.010) - 0.013 = 0.122$$

This gives $(\Delta V)_{\%(corr.)}$ at conditions of T_i and p_i for a 1.063-gram sample. The buret scale reads per cent carbon correctly only for a 1-gram sample at 20° C. and 760 mm. of mercury. A further correction is therefore necessary and may readily be made by substituting, in Equation 6, the appropriate correction factor as obtained from the table supplied with the Lindberg instruction booklet (*5*).

$$\%\text{ C} = \frac{(\Delta V)_{\%(corr.)}}{\text{grams of sample}}(\text{correction factor}) \tag{6}$$
$$= \left(\frac{0.122}{1.063}\right)(0.9408) = 0.108$$

The table is arranged with barometric pressure increments of 5 mm. and temperature increments of 1° C. If one does not wish to interpolate to get values of the correction factor for intermediate values of P and t, Equation 7, which has been fitted to the data of the table, may be used:

$$\text{Correction factor} = (0.3858)\left(\frac{p_{mm.}}{T° \text{ K.}}\right) \tag{7}$$

$$p = (P - \Delta p_s) \tag{8}$$

in which P = barometric pressure at 0° C.

Table I. Results of Analysis of Standard Steels

Sample	Sample Weight, Grams	t_i, ° C.	P, Mm.[a]	Carbon, % Certified value	Carbon, % Found	$(\Delta V)_{\%(corr.)}$ Expected value[b]	$(\Delta V)_{\%(corr.)}$ Found	Diff.
NBS 8g, Bessemer	1.021	24.8	752.79	0.069	0.066	0.073	0.070	−0.003
	1.100	24.0	747.21		0.067	0.079	0.076	−0.003
	1.079	24.5	741.47		0.072	0.078	0.082	+0.004
NBS 16c, BOH	1.016	25.6	753.09	1.01$_5$	1.017	1.071	1.073	+0.002
NBS 55b, ingot iron	1.072	25.0	753.09	0.011	0.015	0.012	0.017	+0.005
	1.082	24.4	749.41		0.004	0.012	0.005	−0.007
	1.049	25.0	747.28		0.013	0.012	0.014	+0.002
Composite NBS 55b NBS 16c	1.017 0.0454	25.0	746.58	0.053	0.050	0.059	0.055$_5$	−0.004
NBS 166, stainless	1.118	27.2	760.51	0.027	0.025	0.031	0.029	+0.002
NBS 101C, 18Cr-9Ni	1.034	26.9	743.57	0.072	0.069	0.079	0.075	−0.004
NBS 15e, BOH	1.115	27.1	760.71	0.107	0.098	0.124	0.113	−0.011
	1.041	24.7	747.71		0.105	0.116	0.114	−0.002
	1.096	24.3	747.88		0.108	0.122	0.123	+0.001
	1.098	30.2	742.42		0.109	0.127	0.129	+0.002
	1.063	27.3	742.57		0.106	0.121	0.120	−0.001
NBS 19e, AOH	1.001	26.1	749.30	0.197	0.195	0.206	0.204	−0.002
	1.027	29.0	748.00		0.205	0.215	0.224	+0.009
	1.047	25.0	744.06		0.205	0.216	0.225	+0.009
NBS 15b, Cr-Ni-Mo	1.042	27.5	760.31	0.515	0.519	0.557	0.562	+0.005

$$S = \sqrt{\frac{\Sigma(\text{diff.})^2}{N}} = 0.005$$

[a] Barometric pressure corrected to 0° C.
[b] Calculated from Equation 6 using certified value of per cent carbon.

On the other hand, the total charge to be burned in the induction furnace should always be about 1 gram. It is therefore necessary, when analyzing samples containing more than 1% carbon, to add to them enough material from a known, low-carbon steel to bring the total charge to 1 gram. When running such a composite sample the procedure and calculations are the same as for a homogeneous sample up to Equation 6. Equation 6 must be modified as shown in Equation 13.

$$\% \text{ C unknown} = \frac{(\Delta V)_{\%(corr.)}\ (\text{correction factor}) - (\text{grams std.})\ (\%\ \text{C std.})}{\text{grams unknown}} \qquad (13)$$

$$\Delta p_s = \text{vapor pressure of water at } T^\circ \text{ K.} - 17.54 \qquad (9)$$

In the illustrative example

$$\% \text{ C} = \left(\frac{0.122}{1.063}\right)(0.3858)\left(\frac{742.57 - [27.21 - 17.54]}{300.5}\right) = 0.108$$

Simplified Calculation. An examination of the data from a large number of determinations has led to the adoption of a simplified calculation of $(\Delta V_f)_{\%}$ which may be used, under the following conditions, as a substitute for the exact equations described previously.

The initial and final values of the barometric pressure are the same and fall in the range 740 to 760 mm. of mercury.

The initial and final values of the temperature, although different, fall in the range 26° to 32° C. The equations to be used are

$$(\Delta V_f)_{\%} = (F_{Tf})(T_f - T_i) \qquad (10)$$

and

$$(F_{Tf}) = F_{30} + (0.003)(T_f - 30.00) \qquad (11)$$

The value of F_{30} to be used in Equation 11 will depend upon the value of the final buret reading, as shown below:

Final Buret Reading, %	F_{30}
Less than 0.7	0.1600
Greater than 0.7	0.1550

The simplified equations will give values for $(\Delta V_f)_{\%}$ which differ by no more than 0.002 from the values obtained from the exact equations. Using the data of the illustrative example:

$$F_{Tf} = 0.1600 + (0.003)(27.20 - 30.00) = 0.1516$$

$$(\Delta V_f)_{\%} = (0.1516)(27.20 - 27.30) = -0.015\%$$

ANALYSIS OF SAMPLES CONTAINING MORE THAN 1% CARBON

The calibrated portion of the buret has a volume of about 25 cc., so that samples which on combustion will produce more than 25 cc. of carbon dioxide under the conditions of measurement cannot be analyzed. A safe rule of thumb for samples containing more than 1% carbon is given in Equation 12.

$$(\text{Grams of sample}) \leq \frac{1}{\%\ \text{C}} \qquad (12)$$

UNCERTAINTY LIMITS OF ANALYSES

The quantity being measured in the analysis is the volume of carbon dioxide in a gas mixture resulting from the combustion of a carbon containing compound. Estimations of the accuracy and precision of the method must therefore be made by burning standard samples and comparing the experimental $(\Delta V)_{\%(corr.)}$ with the $(\Delta V)_{\%(corr.)}$ which should have been obtained under experimental conditions. The latter $(\Delta V)_{\%(corr.)}$ is directly obtained from Equation 6 because the per cent carbon is known.

As a result of a series of such comparisons, shown in Table I, a standard deviation, s, of 0.005 has been established for the measurement of $(\Delta V)_{\%(corr.)}$. Following the procedure of this laboratory the uncertainty limits to be placed upon any single determination of $(\Delta V)_{\%(corr.)}$ would be $\pm 3s$, or ± 0.015 (*4*). In order to translate this figure into the uncertainty limits for the reported per cent carbon it is necessary to multiply $3s$ by the ratio of the appropriate correction factor (Lindberg table or Equation 7) to the weight of the unknown sample:

$$\pm\% \text{ C} = (\pm 0.015)\left(\frac{\text{correction factor}}{\text{grams unknown}}\right) \qquad (14)$$

If a composite sample is analyzed, the weight to be used in Equation 14 is that of the unknown portion, not the weight of the entire charge.

ACKNOWLEDGMENT

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Combustion of Tungsten Carbide by High Frequency Induced Radiant Heating

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Tungsten carbide can be burned rapidly and completely at about 1400° C. in a stream of oxygen without the use of flux. This temperature is attained by subjecting the sample, contained in a small porcelain crucible, to the radiation from a surrounding platinum cage which is heated to about 1600° C. by the high frequency field of a modified Lindberg induction furnace. The resulting carbon dioxide is measured in the Lindberg volumetric apparatus. The standard deviation for the determination of the carbon in a tungsten carbide sample is 0.033% carbon.

THE carbon content of tungsten carbide is determined by measuring the carbon dioxide produced when a sample of the material is burned in oxygen. At the temperatures readily attainable in the conventional laboratory furnaces (1000° to 1100° C.) complete combustion is effected only in the presence of a flux, and after an oxidation period of 10 to 15 minutes (*2*). The authors have been able to burn tungsten carbide rapidly and completely at about 1400° C. in a stream of oxygen without the use of flux by subjecting the sample, contained in a small porcelain crucible, to the radiation from a surrounding platinum cage which was heated to about 1600° C. by the high frequency field of a modified Lindberg induction furnace. The resulting carbon dioxide was measured in the Lindberg volumetric apparatus. The standard deviation for the determination of the carbon in a tungsten carbide sample was 0.033% carbon.

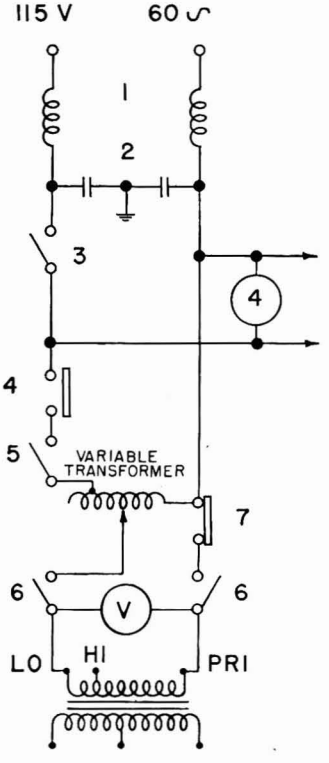

Figure 1. Modified portion of electrical circuit of Lindberg high frequency induction furnace

EXPERIMENTAL

The apparatus used in this investigation was the high frequency combustion furnace (LI-500-A) and volumetric attachment, manufactured by the Lindberg Engineering Co. and modified as described in this paper.

The initial combustion experiments were carried out with about 100 mg. of carbide mixed with about 1 gram of known low-carbon steel and 0.25 gram of tin to provide a matrix which would couple with the high frequency field. The scatter of the analytical results was great, and most of the runs led to carbon values lower than the stoichiometric value of 6.13%. Attempts to use tin alone or auxiliary fluxes such as vanadium or copper oxides proved equally unsuccessful.

The use of fluxes was abandoned, and the Lindberg crucible was wrapped in platinum foil to permit the sample container to couple directly with the high frequency field. The coupling of the field with the foil was so strong that the platinum was fused almost instantly. By trial and error, satisfactory combustion without damage to the platinum was achieved by abandoning the regular Lindberg crucible and using a Coors 5/0 porcelain crucible sandwiched between two disks of 40-mil platinum ($^{13}/_{16}$-inch diameter), the upper one having a 0.25-inch hole in its center. (These crucibles were cleaned before use by boiling for 5 to 10 minutes in concentrated nitric acid and then igniting for 2 hours in a muffle furnace at 900° C.)

By means of optical pyrometry the temperature of the upper platinum disk during operation of the unit was estimated to be 1600° C. Under the conditions it was possible to fuse a sample of calcium fluoride (melting point 1386° C.) but not a sample of nickel (melting point 1453° C.) in the crucible. The temperature achieved by radiation inside the crucible is therefore about 1400° C. The geometry of the platinum radiators proved to be critical, and a number of test specimens of radiators were melted before the design described above was developed.

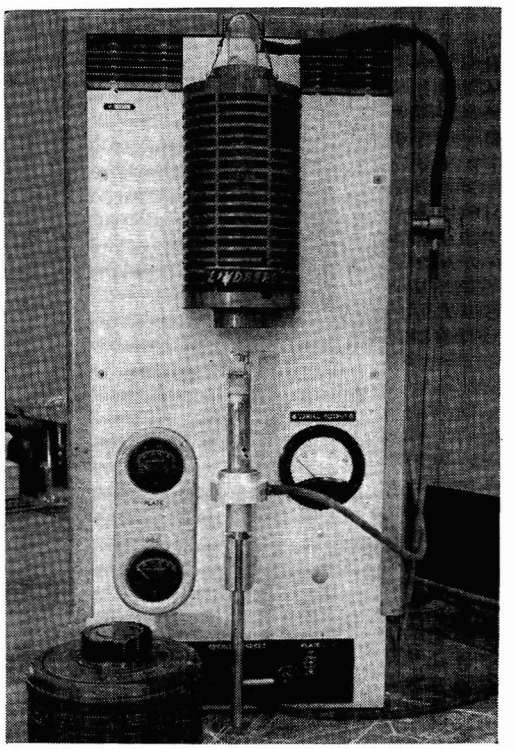

Figure 2. Modified Lindberg high frequency induction furnace

In order to achieve greater flexibility in the use of the apparatus, the electrical circuit was modified to permit the operator to control the power output of the oscillator. This was done by introducing a variable transformer in the input circuit so as to control the high voltage supplied to the rectifier tube (see Figure 1). The output voltage from the variable transformer was measured by means of the voltmeter, also shown in Figure 1. A photo-

graph of the modified unit is shown in Figure 2. Complete wiring diagram is shown by the manufacturer (*5*).

With this arrangement it is possible, for a chosen design of radiator, to regulate the power supply as necessary to achieve the desired temperature. The proper setting for the variable transformer must be determined in a trial experiment in which the platinum temperature is measured with an optical pyrometer at various voltage settings. The maximum temperature is, of course, limited by the melting point of the radiator material (1769° C. in the case of platinum). For any given voltage setting and radiator design, the temperature in the crucible can be estimated from the melting behavior of various substances of known melting point.

The radiator design currently in use in this laboratory is shown in Figure 3. It consists of two 40-mil platinum disks ($^{13}/_{16}$-inch diameter) connected by three channel-type posts built from 20-mil platinum sheet and spot welded at top and bottom. The 5/0 crucible which contains the sample rests on the bottom of the cage. With the present design a temperature of about 1400° C. inside the crucible can be produced when the variable transformer output is 118 volts.

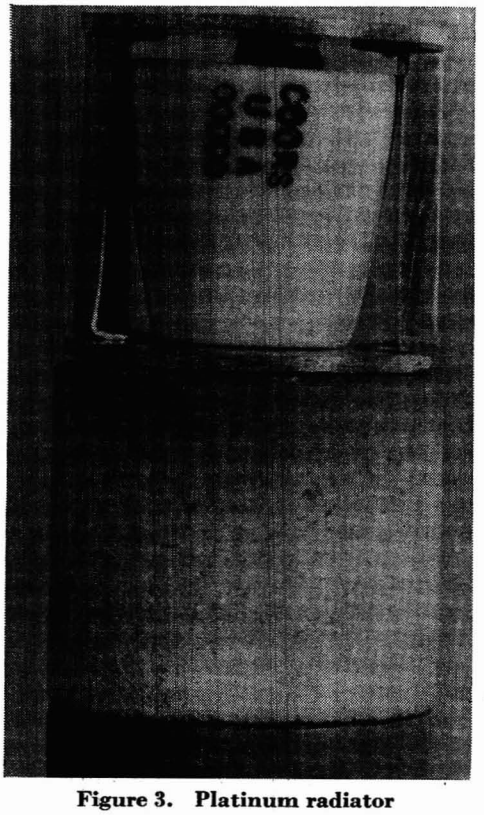

Figure 3. Platinum radiator

Procedure. The operation of the furnace is essentially the same as that described by the manufacturers (*5*). To start a combustion run the sample crucible and radiator cage are raised into position in the combustion chamber, the filament switch is turned on, and the variable transformer is set at zero voltage. After the filament has warmed up, the plate switch is turned on, the variable transformer is turned up until the voltmeter registers the desired output, and the oxygen flow is begun at a rate of about 150 cc. per minute. Under these conditions combustion is complete, and the Lindberg gas buret is filled in about 3.5 minutes. With a transformer output of 118 volts, the initial plate current is about 270 ma. and rises during the combustion to a value of about 310 ma. The grid current varies between 50 and 60 ma.

RESULTS

This combustion method was tested on a tungsten carbide sample supplied by the Carboloy Department of the General Electric Co. The combustion was carried out as described above, and the carbon dioxide was measured in the Lindberg volumetric apparatus, using the modifications and precautions described elsewhere (*6*). The data from 26 determinations are listed in Table I. The average value obtained was 6.24% carbon, with a standard deviation of 0.033% carbon. Following the practice of this laboratory, the result of a single determination can be guaranteed within 3*s* limits, or ±0.10% carbon; the average of n determinations can be guaranteed within $\pm 0.10/\sqrt{n}$ % carbon (*4*).

DISCUSSION

In the absence of an absolute value for the carbon content of this sample, the evidence that the method described in this report has resulted in complete combustion can only be indirect.

The standard deviation obtained in this series of determinations may be compared with that which would have been expected for the determination of the same amount of carbon in a steel sample for which combustion is complete. As reported previously (*6*), the standard deviation for the carbon determination on a 1-gram steel sample is 0.005% carbon. The standard deviation in per cent carbon for a sample of any other weight is

$$s_{\%C} = (0.005)\,\frac{\text{(corr. factor for } T \text{ and } p)}{\text{(grams of sample)}}$$

The average sample weight for the 26 determinations listed in Table I was 0.1474 gram and the average correction factor was 0.95325. The expected standard deviation, therefore, is 0.032% carbon, which is in good agreement with the experimentally determined value of 0.033% carbon.

Table I. Determination of Carbon in Tungsten Carbide

Sample Wt., Mg.	Carbon, %	Sample Wt., Mg.	Carbon, %
148.6	6.26	142.0	6.23
149.5	6.22	134.0	6.24
139.0	6.29	137.6	6.24
140.0	6.26	151.8	6.23
144.0	6.23	141.2	6.29
142.1	6.27	156.5	6.21
139.8	6.34	158.5	6.21
144.1	6.25	157.5	6.20
141.9	6.27	153.5	6.20
154.2	6.20	151.4	6.27
153.1	6.21	159.2	6.22
143.7	6.20	156.5	6.20
143.0	6.29	153.0	6.29
			Av. 6.24

$$s = \sqrt{\frac{\Sigma\,(x - \bar{x})^2}{n - 1}}$$

$n = 26$

$s = 0.033$

Blank runs made on empty crucibles produced no carbon dioxide.

Five 200-mg. samples of a standard steel (NBC 16c-1.01$_5$% carbon) were burned by exactly the same technique as that used in the combustion of tungsten carbide. The 200-mg. samples of steel were too small to couple directly with the field in the Lindberg high frequency furnace. They were burned by radiant heating just as carbide is burned in this method. The results of the five runs show the absence of any bias in the method:

% C
1.003
1.048
1.015
0.990
0.983
Av. 1.008

By carrying out the combustion of the tungsten carbide at temperatures lower than about 1400° C. (transformer output less than 118 volts), results as low as 4% carbon could be obtained. The tungsten oxide residues from these runs, when reburned at 1400° C. with about 50 mg. of vanadium pentoxide in the cruci-

ble as a flux, produced carbon dioxide. The oxide residues from the 1400° C. combustions (6.24% carbon) produced no additional carbon dioxide when reburned in the presence of vanadium pentoxide.

The gaseous combustion products from several runs, both complete and incomplete combustion, were examined with a Mine Safety Appliances monoxide detector. No carbon monoxide was noted in any cases.

The stoichiometric value for tungsten carbide is 6.13% carbon; this sample, therefore, contains an excess of 0.11% carbon.

The principle of high frequency induced radiant heating is not new. In this laboratory Horn and Neubauer (*3*) have used a quartz crucible whose hollow walls were filled with a low melting alloy as a container for melting silicon in a high frequency field. More recently, Bennnet (*1*) has described a quartz-enclosed carbon crucible which he has used for the combustion of noncoupling samples in a Leco (Laboratory Equipment Co.) high frequency furnace.

The authors believe that for routine combustion work the platinum cage coupler, developed independently here, has two advantages over the quartz-enclosed graphite crucible: It is less fragile, and it provides a relatively large radiator surface directly over the mouth of the sample crucible.

ACKNOWLEDGMENT

The authors wish to thank Marie De Vito for her assistance in combustion work.

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Determination of Magnesium in Alkali Products

Photometric Method Using Thiazole Yellow

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A rapid and precise method for the determination of less than 0.1% magnesium in alkali products was required. The use of thiazole yellow to determine magnesium as a magnesium dye lake in a sodium chloride solution has been developed. Results are accurate to within 2 to 5% of the true value. The critical factors of magnesium dye lake stability, removal of interfering cations, and the control of sodium chloride concentration were studied thoroughly in respect to their effect on color development.

THE colorimetric determination of magnesium with thiazole yellow has been used principally in the analysis of plant tissue and soil extracts (*2*, *5*, *6*). Mikkelsen and Toth (*3*) found thiazole yellow (sodium salt of 2,2-disulfonate of methyl benzothiazole) superior as a reagent for magnesium. The use of thiazole yellow for the determination of micro amounts of magnesium in alkali products has not been previously reported. A number of texts refer to thiazole yellow as being synonymous with Titan yellow, Clayton yellow, mimosa, etc. Thiazole yellow (Eastman P5977) was used in this work.

The proposed procedure was developed in three steps: (1), the removal of aluminum, copper, iron, manganese, and nickel through the chloroform extraction of their oxinates; (2), the removal of calcium and the decrease in sodium chloride concentration by treatment with sulfuric acid in a 98% methanol solution; and (3), the formation of a stabilized magnesium dye lake.

This paper describes the factors which influence the development and stability of the magnesium dye lake complex in a sodium chloride solution. The factors investigated were: reagent concentration, effect of light and heat, the stabilization of the thiazole yellow reagent and magnesium dye lake, the effect of interfering cations and their removal, the effect of various concentrations of sodium chloride, the procedure for producing a nearly constant low concentration of sodium chloride, and the reproducibility of the method.

APPARATUS AND REAGENTS

Beckman spectrophotometer, Model DU, with two 1-cm. matched cells.

Beckman pH meter, Model H-2.

Amber mixing cylinders, 50-ml.

A water bath at 60° to 70° C. consisting of a variable controlled electric hot plate and a 150-mm. borosilicate glass crystallizing dish partially filled with water.

A water bath in the sink maintained at 25° C. by proper regulation of the hot and cold water streams.

Alcohol, Formula 30.

Chloroform, reagent grade.

Hydrochloric acid, 10.0*N*.

Hydroxylamine hydrochloride, 5% w./v.

Methanol, 99.5%, reagent grade.

Sodium chloride recrystallized twice.

Sodium hydroxide, 1.0*N*.

Sodium hydroxide, 10.0*N* (prepared from mercury cell caustic soda).

Sulfuric acid, 9.0*N*.

8-Hydroxyquinoline (oxine 1.2% w./v.). Dissolve 2 grams of oxine (Eastman 794) in 6 ml. of glacial acetic acid and dilute to 100 ml. with water.

Poly(vinyl alcohol), 2% w./v. Dissolve 20 grams of poly(vinyl alcohol) (Du Pont Elvanol Grade 71-24) in 400 ml. of water using heat up to 90° C., and stirring. Dilute the cool solution to 1 liter with water and store in the refrigerator.

Poly(vinyl alcohol), 0.5% w./v. Dilute 50 ml. of 2% poly(vinyl alcohol) to 200 ml. with water.

Thiazole yellow, 0.5% w./v. Dissolve 0.5 gram of thiazole yellow (Eastman P5977) in 50 ml. of 95% ethyl alcohol and dilute to 100 ml. with water. This reagent will keep indefinitely when stored in a dark bottle.

Thiazole yellow, 0.01% w./v. Add 2 ml. of 0.5% thiazole yellow and 5 ml. of 0.5% poly(vinyl alcohol) to water, and dilute with water to 100 ml. A fresh 0.01% thiazole yellow solution should be prepared at least once every 2 weeks and stored in a dark bottle.

Standard magnesium stock solution (1 ml. contains 5.0 mg. of

magnesium oxide). Dissolve 3.016 grams of magnesium metal in 50 ml. of 1 to 1 hydrochloric acid. Dilute this solution to 1 liter with 50 ml. of concentrated hydrochloric acid and water.

Standard magnesium working solution (1 ml. contains 20γ of magnesium oxide). Pipet 4 ml. of magnesium stock solution into a 1-liter flask, and dilute to the mark with water.

RECOMMENDED PROCEDURE

Sample Preparation. The following sample weights should be used for analysis.

Sample	Weight, Grams
Sodium bicarbonate	160
Sodium carbonate	100
Sodium chloride	115
Sodium sesquicarbonate	140
Sodium hydroxide	
98 to 100%	75
70%	100
50%	150

Except for sodium chloride, dissolve the indicated weight of sample in about 250 ml. of water. Neutralize the solution by slowly adding 200 ml. of 10.0N hydrochloric acid and dilute to 500 ml. with water. For sodium chloride, dissolve the indicated weight in water and dilute to 500 ml.

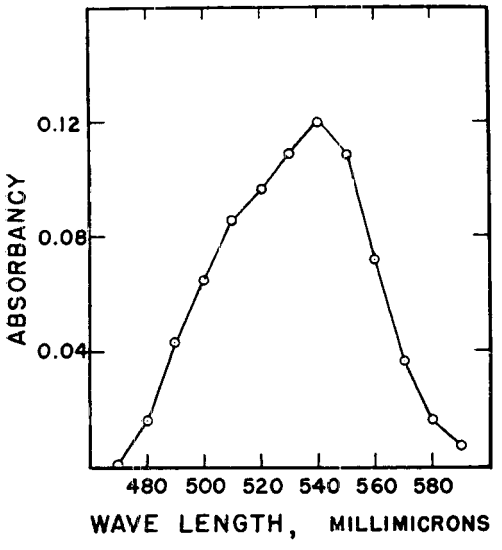

Figure 1. Absorption spectrum

Net absorbancy of 100 γ of magnesium oxide in the presence of 0.58 gram of sodium chloride

Preliminary Treatment. Place a 25-ml. aliquot of the sample, containing approximately 5.8 grams of sodium chloride and from 20 to 200 γ of magnesium oxide, in a 150-ml. beaker. Add 2 ml. of 2% oxine, and adjust the pH of the solution to 7.2 using 1.0N sodium hydroxide and a pH meter. Digest the sample 15 minutes in a water bath at 60° to 70° C., cool in an ice bath, and transfer to a 150-ml. separatory funnel. Adjust the sample volume to 80 ml. with water.

Add 10 ml. of chloroform and shake for 2 minutes. On separation, remove and discard the chloroform layer. The chloroform extracts the metallic oxinates of aluminum, copper, iron, nickel, and manganese as well as the unused oxine. Make a second extraction with 10 ml. of chloroform, shaking the sample 1 minute and discarding the chloroform layer. Add 5 drops of concentrated hydrochloric acid to the aqueous layer to lower the pH below 4 and keep the magnesium in solution. Transfer the aqueous layer to the original beaker and evaporate to dryness.

Add 1 ml. of 9.0N sulfuric acid and 50 ml. of 99.5% methanol to the cool dry sample. Let the slurry of salts stand 30 minutes, filter, and evaporate the methanol filtrate to dryness. This step reduces the salt content to 0.30 to 0.75 gram and eliminates interfering quantities of calcium. Redissolve the dry sample in 12 ml. of water and 5 ml. of alcohol (Formula 30), filter into a 50-ml. amber mixing cylinder, and make up to 30 ml. with water.

Magnesium Determination. To the sample in the mixing cylinder add 5 ml. of 5% hydroxylamine hydrochloride, 4 ml. of 0.5% poly(vinyl alcohol), and 5 ml. of 0.01% thiazole yellow reagent. Adjust the volume to 45 ml. with water and let stand in a water bath at 25° C. ± 0.5° for 10 minutes. Add 3.5 ml. of 10.0N sodium hydroxide, adjust the volume to 50 ml. with water, and let stand 15 minutes in the water bath at 25° C. ± 0.5° for complete color development.

Within 30 minutes after the color is completely developed, measure the absorbancy of the sample in a 1-cm. cell at 540 mμ with the spectrophotometer previously adjusted to zero absorbancy with distilled water. A reagent blank containing 0.58 gram of twice recrystallized sodium chloride should be processed according to the magnesium determination and the absorbancy determined. Subtract the reagent blank absorbancy from the sample absorbancy and determine the micrograms of magnesium oxide by consulting a standard curve. The average value of duplicate samples is used to improve the accuracy of the method.

A standard curve was prepared by using solutions containing 0.58 gram of twice recrystallized sodium chloride plus suitable aliquots of the standard magnesium working solution, and then following the magnesium determination without preliminary treatment.

EXPERIMENTAL

In previously reported work the absorbancy of the magnesium dye lake has been measured at 540 mμ using filter photometers. The spectrum of the magnesium dye lake in the presence of 0.58 gram of sodium chloride was prepared from 470 to 590 mμ (Figure 1). All values were corrected by subtracting the reagent blank absorbancy from the observed readings. Since the highest sample absorbancy value occurred at 540 mμ, this wave length was used throughout the work.

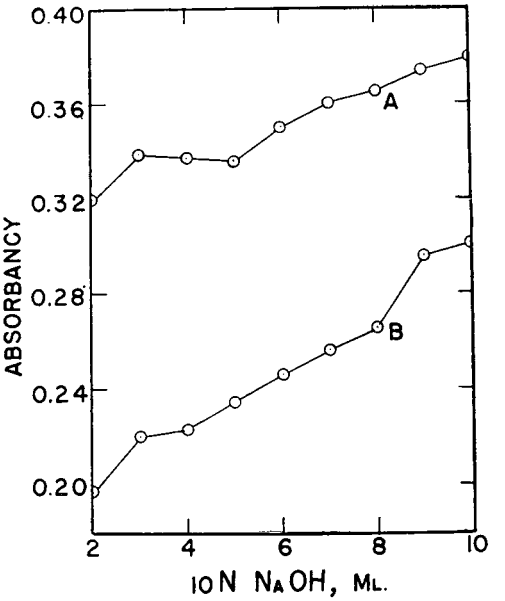

Figure 2. Variation of sodium hydroxide concentration

A. **Magnesium oxide, 100 γ**
B. **Reagent blank**

The initial work in this investigation incorporated several of the reagents and techniques used by Young and Gill (*6*) in their analysis of plant tissue for magnesium. Since the sodium chloride solution, formed as the neutralization product of various alkali samples, contained variable amounts of aluminum, calcium, copper, iron, manganese, and nickel, it was considered more practical to remove these constituents than to control the level

of them through the use of a compensatory solution as many previous workers have done. The addition of a compensatory solution to a sodium chloride solution would increase the total salt concentration and could thereby adversely influence the formation of the magnesium dye lake.

Stability of Magnesium Dye Lake. The use of hydroxylamine hydrochloride and poly(vinyl alcohol) was found necessary to attain color-complex stability. Five milliliters of 5% hydroxylamine hydrochloride were required to provide adequate protection against oxidants which may impede color formation or induce color fading.

Young and Gill (*6*) have reported that stabilizing agents such as starch and gum ghatti proved to be unsatisfactory because colored solutions prepared with them lacked sensitivity and reproducibility. The degree of color reproducibility is dependent upon the formation of uniformly sized particles of magnesium hydroxide as a colloidal suspension and the adsorption of thiazole yellow upon these particles to form the rose-colored magnesium dye lake. Any variation of conditions which will influence the particle size of the magnesium hydroxide will in turn affect the magnesium dye lake formed. For this reason a stabilizing agent is required to control the formation of magnesium hydroxide. Young and Gill attained greater sensitivity and stability through the use of poly(vinyl alcohol) and a higher alkali concentration. Experimentally, the least variation in absorbancy values (less than 1% over a 60-minute period) for reagent blanks and magnesium samples, both with and without sodium chloride, was attained by using 4 ml. of 0.5% poly(vinyl alcohol).

Concentration and Stability of Thiazole Yellow Reagent. The stock 0.5% thiazole yellow solution was prepared in 50% alcohol as recommended by Young and Gill (*6*). This solution will keep indefinitely. A more dilute (0.01%) thiazole yellow reagent was prepared to permit more accurate volumetric measurements and greater precision of results. However, this weak solution decreased in strength daily unless the reagent was stabilized by the addition of 5 ml. of 0.5% poly(vinyl alcohol) in each 100 ml. of reagent. The use of poly(vinyl alcohol) to stabilize the thiazole reagent has not been previously reported. By its use, the dilute reagent could be kept for 2 weeks if stored in an amber bottle.

The optimum concentration of reagent was found to be 5 ml. of 0.01% thiazole yellow per 50 ml. of sample solution. This gave the most consistent results and the highest absorbancy.

Addition of Reagents. The three reagents [hydroxylamine hydrochloride, poly(vinyl alcohol), and 0.01% thiazole yellow] may be added in succession to the sample without any time interval.

Concentration of Sodium Hydroxide. The concentration of sodium hydroxide has an important bearing upon the stability and degree of color intensity in the complex formed, as it is the precipitating agent for the magnesium. The absorbancy values for both a reagent blank and a sample (containing no salt and treated with 6 ml. of 10.0*N* sodium hydroxide) shifted in a similar manner to give a nearly constant net absorbancy over the 45-minute test period. Higher sodium hydroxide concentrations produced greater absorbancy increases in the blank than in the sample.

However, in the presence of sodium chloride (2.3 grams), samples treated with 3 to 5 ml. of 10.0*N* sodium hydroxide had no appreciable variation in absorbancy over a 45-minute period. A more critical study of the influence of variations in the sodium hydroxide concentration upon reagent blanks and samples containing 0.58 gram of sodium chloride (the sodium chloride concentration at which the thiazole yellow complex is developed) was made (Figure 2). The photometer was standardized against water for both the blank and the sample and absorbancy values were determined about 30 minutes after the addition of the last reagent. The least net absorbancy variation was noted when 3 to 4 ml. of 10.0*N* sodium hydroxide were used. A concentration of 3.5 ml. of 10.0*N* sodium hydroxide was chosen for this work.

Effect of Light and Temperature. Both strong light and variations of temperature influenced the intensity of color formed and its stability. Strong light caused color fading which was overcome by using amber mixing cylinders. Slight differences of temperature influenced the intensity of the color complex formed, as will be noted in Table I. For this reason samples were placed in a water bath at 25° C. before and during the period of color development.

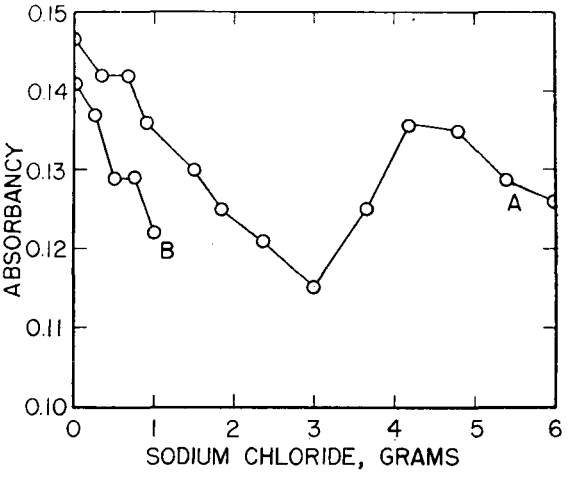

Figure 3. Variation of sodium chloride concentration

Magnesium oxide, 100 γ in each sample
***A.* No preliminary sample treatment**
***B.* Trace metals removed with oxine**

Effect of Sodium Chloride on Color Complex. Variations in the concentration of sodium chloride were found to have an appreciable effect upon the formation of the color complex. The analyses of samples containing 165 γ of magnesium oxide and 2.64 to 2.91 grams of sodium chloride had an absorbancy range from 0.181 to 0.157. More extensive data were procured from the analyses of samples containing 100 γ of magnesium oxide and from 0 to 6 grams of sodium chloride. This information is found on curve *A*, Figure 3. The samples used to obtain data for curve *B* on Figure 3 were given the preliminary oxine treatment to remove aluminum, copper, iron, and nickel that might be present. On both curves there are flat ranges between 0.30 and 0.75 gram of sodium chloride which nearly coincide. In the procedure described later, to remove calcium and decrease the sodium chloride concentration, a series of ten samples analyzed contained between 0.45 and 0.61 gram of total salts. In the recommended procedure the sodium chloride concentration is reduced to the 0.30 to 0.75-gram level to overcome the effect of slight variations in the chloride ion.

Table I. Effect of Temperature

MgO, γ	° C.	Absorbancy
132	20	0.080
132	25	0.104
132	30	0.116

According to Seidell (*4*) the solubility of magnesium hydroxide in water is 0.000168 gram-mole per liter at 18° C. The presence of sodium chloride or sodium sulfate increases this solubility. The solubility factor explained why the presence of sodium

chloride was critical and the lower limit of the standard curve was established at 20 γ of magnesium oxide.

Removal of Calcium and Reduction of Sodium Chloride Concentration. Samples to be analyzed may contain calcium in sufficient quantity to cause possible interference. An investigation of the effect of calcium on the magnesium determination revealed that 0 to 5 mg. of calcium oxide had no effect while larger amounts produced turbid solutions. To remove calcium and at the same time reduce the concentration of sodium chloride, the procedure described by Caley and Elving (*1*) was modified by using 1 ml. of 9*N* sulfuric acid in 50 ml. of 99.5% methanol. After standing 30 minutes the slurry of salts was filtered and the filtrate was evaporated to remove the alcohol. The residue contained the magnesium and 0.50 ± 0.2 gram of sodium salts. This technique produced the desirable level of sodium chloride.

Interfering Metals and Their Removal. Aluminum, copper, iron, manganese, nickel, and strontium affected the magnesium determination in varying degrees and in diverse ways. The effect of these constituents individually is best illustrated by Figure 4. The absorbancy of magnesium was greatly increased by the presence of 15 to 25 γ of copper. Process liquors and samples have variable amounts of copper which would require its removal before the analysis of the sample. Strontium had little or no effect upon the magnesium determination and could be disregarded in most cases.

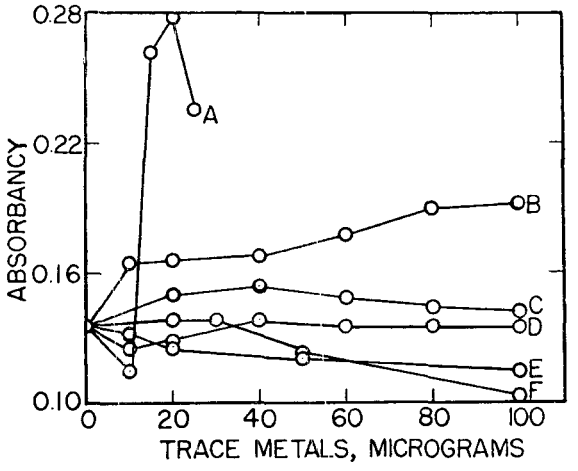

Figure 4. Effect of trace metals on magnesium determination

Magnesium oxide, 100 γ in each sample

A. **Copper**
B. **Manganese**
C. **Nickel**
D. **Strontium**
E. **Aluminum oxide**
F. **Ferric oxide**

Aluminum, copper, iron, manganese, and nickel were removed by the chloroform extraction of their oxinates at pH 7.2. To ensure complete recovery of magnesium, the pH of the aqueous layer had to be decreased to 4 with hydrochloric acid. Magnesium is frequently precipitated as magnesium oxinate for determination in macro quantities. No published data were found concerning the solubility of micro amounts of magnesium oxinate in chloroform. Experimental tests made on 40, 100, and 200 γ of magnesium oxide at pH 7.2 gave no evidence that magnesium oxinate had been dissolved in the chloroform extract.

In other exploratory tests the determination of magnesium was unaffected by the presence of 0.245 gram of sulfate ion, 240 γ of silica, 10 γ of vanadium, or 50 γ of ammonia. Larger amounts of sulfate produced turbidity in both reagent blank and sample. Silica will be removed in the preliminary treatment of the sample, thereby eliminating any interference. Traces of silver, frequently found in reagent grade sodium chloride, strongly decreased the absorbancy of the magnesium dye lake.

Table II. Reproducibility

Sample	Instrument Reading	Blank	Net Absorbancy
1	0.305	0.205	0.100
2	0.303	0.205	0.098
3	0.322	0.225	0.097
4	0.320	0.225	0.095
5	0.318	0.221	0.097
6	0.315	0.221	0.094
Av.	...	...	0.097

Reproducibility. The data on the reproducibility of this method are presented in Table II. A series of synthetic samples containing 100 γ of magnesium oxide, 2.92 grams of sodium chloride, and 50 mg. of calcium oxide were analyzed according to the recommended procedure. In the set of six samples the results varied ±3% from the arithmetic mean. Known amounts of magnesium were added to another set of eight brine samples and the magnesium was recovered within 4 to 5% of the initial value. These data are found in Table III.

Table III. Recovery of Added Magnesium

Sample	MgO Added, γ	MgO Found, γ
1	20	20
2	20	20
3	20	22
4	20	22
Average of above four samples		21
5	50	49.5
6	50	49.5
7	50	53.0
8	50	46.0
Average of above four samples		49.5

Experimental Data on Alkali Products. Samples of raw and purified brine, sodium carbonate, sodium bicarbonate, flake caustic soda, and 50% caustic soda liquor have been analyzed by the previously described procedure. The data are found in Table IV.

Table IV. Analysis of Some Alkali Products and Raw Materials

Sample	MgO Found, P.P.M.
1. Raw brine (22.5 grams/liter)	4.2, 4.2
2. Raw brine (27.2 grams/liter)	4.2. 4.9
3. Raw brine (31.8 grams/liter)	5.4, 5.4
4. Raw brine (125.1 grams/liter)	17.6, 17.5
5. Purified brine, saturated	1.8, 1.4
6. Sodium carbonate	19.0, 19.4
7. Sodium bicarbonate	18.3, 18.3, 18.5
8. Sodium bicarbonate	17.5, 17.7
9. Flake caustic soda	19.7, 14.9
10. 50% caustic soda liquor	4.9, 4.5
11. 50% caustic soda liquor	2.7, 2.3

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Paper Chromatography of Nicotine and Related Compounds

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In the course of an investigation of the constituents of tobacco, improved separations of tobacco alkaloids and related materials by paper chromatography were achieved by pretreatment of the paper before use. Spraying the sheet with pH 5.6 acetate buffer is recommended, although the improvement resulting from pretreatment is not regarded as due only to pH effects. Such treatment improves the symmetry of spots and allows less variation of R_f with solute concentration. The distribution of R_f values obtained by the recommended method appears to be highly suited to screening compounds present in tobacco, tobacco extracts, and smoke with respect to nicotine and allied materials.

RECENTLY, Porter, Naghski, and Eisner (*2*), Werle and Koch (*5*), Tso and Jeffrey (*3*), Kraft (*1*), and Wegner (*4*) have used paper chromatography for the separation and identification of the tobacco alkaloids and related compounds. Porter, Naghski, and Eisner and Tso and Jeffrey recognized the advantage of controlling the pH of the developing solvent. Kraft and Wegner found that pretreatment of the paper by dipping it in complex ion buffers, such as citrate and phosphate, was helpful in separating nicotine, nornicotine, and anabasine. However, the separation of complex mixtures of alkaloids by paper chromatography is not easy. Elongated spots, closely similar R_f values, and the tendency of R_f values to depend on the concentration of the alkaloid present are the principal difficulties. Furthermore, the separations are influenced by variables in regard to paper, developer, sample, and experimental conditions, making known controls a necessity. It has been found that the difficulties in the separation of the alkaloids on paper can best be overcome by spraying the filter paper lightly with an aqueous pH 5.6 acetate buffer solution before use.

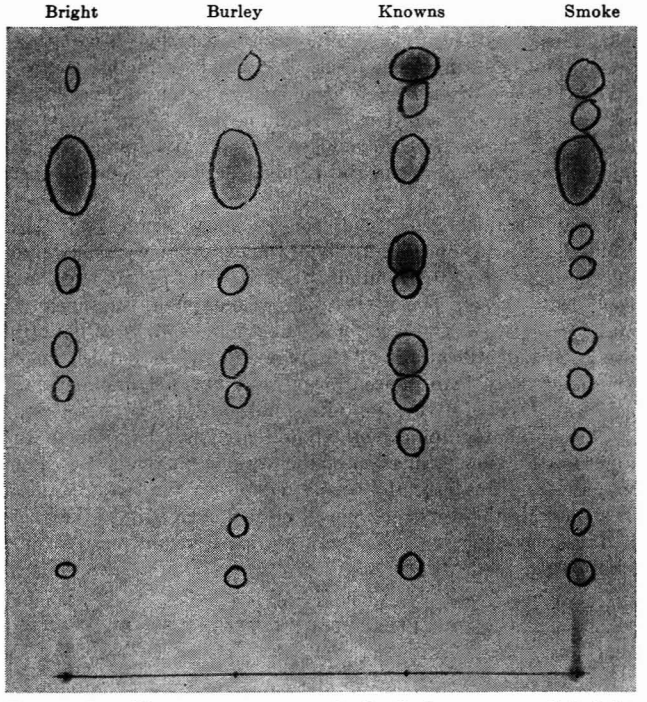

Figure 1. Chromatograms of alcohol extract of bright tobacco, alcohol extract of burley tobacco, synthetic mixture, and sample of cigarette smoke

In the synthetic mixture the knowns, in order of increasing R_f are: nicotinic acid, *N*-methylmyosmine, oxynicotine, nornicotine, anabasine, metanicotine, nicotine, 2-methyl-6-(3′-pyridyl)-tetrahydro-1,2-oxazine, and nicotysine

Standardized Procedure. Whatman No. 1 paper is cut to suitable size, preferably about 11 by 16 inches, and points for application of sample are marked near one end. About 10 ml. of the buffer recommended by Porter, Naghski, and Eisner, prepared by mixing 95 ml. of aqueous 0.2*M* acetic acid and 905 ml. of 0.2*M* sodium acetate solution, is sprayed lightly but evenly over the sheet, which is then allowed to air-dry. After application of about 10 to 50 γ of solutes to the starting point, the sheet is placed in an atmosphere saturated with water vapor for about 30 minutes and then is developed for 16 hours by the ascending method, using 1-butanol saturated with pH 5.6 acetate buffer. This is done at about 74° F. in a closed glass cylinder containing a paper liner dipping in pH 5.6 buffer solution saturated with 1-butanol. The developed chromatogram is air-dried, sprayed with a 1% alcoholic solution of *p*-aminobenzoic acid, air-dried again, and finally dropped into a tank containing cyanogen bromide fumes. Colored spots appear where the alkaloids have been deposited by the developer. R_f values are measured in the usual way, using the point of application of sample as the reference mark for measuring travel of solvent and travel of solute.

The degree of separation normally achieved is demonstrated in Figure 1, a photograph, which shows the resolution of an alcohol extract of bright tobacco, an alcohol extract of burley tobacco, a synthetic mixture containing nicotinic acid, *n*-methylmyosmine, oxynicotine, nornicotine, anabasine, metanicotine, nicotine, 2-methyl-6-(3′-pyridyl)-tetrahydro-1,2-oxazine, and nicotyrine, and a sample of smoke from a cigarette containing a blend of flue-cured, burley, Maryland, and Turkish tobaccos.

Figure 1 indicates the presence of nicotinic acid, oxynicotine, nornicotine, anabasine, and nicotine in the three sample solutions chromatographed. However, tobacco extracts and tobacco smoke contain a family of alkaloids and related materials, some of which may have R_f values similar to the R_f values of the known components of the synthetic mixture. It is wise to refrain from attempted positive identification of materials of this type based on R_f values under a single set of chromatographic conditions. Further, it is known to the authors that an extract of tobacco when chromatographed may not represent exactly what was removed from the tobacco. The composition of some extracts has been observed to change on standing, particularly with formation of oxynicotine and nicotinic acid.

A variation in the standard method is suggested when attention is to be focused on fast-running materials. This variation

Table I. R_f Values of Tobacco Alkaloids and Related Materials

(Averages of 9 runs)

Substance	Liner in Aqueous Phase	Liner in Organic Phase
Nicotyrine	0.93	0.84
Oxazine[a]	0.90	0.85
Nicotine	0.75	0.75
Nicotinamide	0.64	0.59
Metanicotine	0.62	0.55
Anabasine[b]	0.55	0.51
Nornicotine[c]	0.41	0.41
Oxynicotine	0.36	0.33
N-Methylmyosmine	0.31	0.28
Nicotinic acid	0.15	0.12

[a] 2-Methyl-6-(3′-pyridyl)-tetrahydro-1,2-oxazine.
[b] Anabasine from Louis Feinstein, U. S. Dept. Agriculture.
[c] Nornicotine from a *N. sylvestris*–D.B. 101 hybrid.

involves allowing the tank liner to dip into the developing solvent itself, rather than the inverse phase of the developing solvent. With the liner dipping into the aqueous phase of the butanol-buffer mixture, the spots formed by nicotyrine and oxazine are sometimes diffuse and unsymmetrical, apparently because of loss of butanol from the advancing front by evaporation. With the liner dipping into the organic phase, the R_f values for these two materials are lower, and the spots are more concise and symmetrical. The standard method is preferable when interest is on nornicotine, anabasine, and metanicotine.

In Table I are shown R_f values obtained by the standard method along with the values obtained by using 1-butanol saturated with pH 5.6 buffer on the cylinder liner.

DISCUSSION

The addition of a buffer to the developing solvent when separating polar molecules by chromatographic methods is logical. The pH control gained by addition of buffer contributes to the stability of the system, and of course to the reproducibility of the results. The pH of the buffer used is important. In general, with alkaloidal materials, the R_f of the solute is dependent on the pH of the buffer. Further, it appears to be desirable to avoid buffers derived from polybasic acids, because complex combinations of alkaloids and buffer anions may occur, causing odd results. For successful chromatography, the individual molecules of solute must be continually passing back and forth between the mobile phase and the nonmobile phase. If this dynamic equilibrium is upset, by pH changes or otherwise, the resulting spot is not likely to be round.

Tobacco alkaloids chromatographed on untreated paper with a buffered solvent have been observed to give elongated spots. Adsorption of the alkaloids by the paper support might contribute to the elongation of spots. On the other hand, alcohol saturated with an inorganic buffer used as mobile phase on a moist paper sheet support can hardly be regarded as a stable system. One should expect removal of the inorganic buffer salt from the mobile phase by the nonmobile water phase.

Further, when tobacco alkaloids, particularly nicotine, are run at widely different concentrations on an untreated sheet using buffered 1-butanol as solvent, the R_f values vary with concentration. This suggests that not enough buffer is present. It appears logical to incorporate buffer in the nonmobile phase, the water phase on the sheet. By such pretreatment the symmetry of spots is improved and the variation of R_f with concentration of solute is decreased.

It has been demonstrated that the salts applied to the sheet during spraying are immobile during development of the chromatogram. Sheets have been masked during the spraying with buffer so that only the bottom portion of each sheet is sprayed. These sheets have been run in both 1-butanol saturated with water, and 1-butanol saturated with pH 5.6 acetate buffer, no alkaloids being present. In each case, examination of the "developed" and dried sheets by streaking and spraying of pH indicator solutions indicated that the sodium acetate was still uniformly distributed in the area where it had been applied, and had not been carried onto the untreated portion of the sheet by the developer.

The sprayed and air-dried sheet does not have a pH of 5.6 corresponding to the buffer sprayed onto it. Application of pH indicators to the aqueous phase on the air dried sheet shows it to have a pH of about 7, consistent with loss of acetic acid on drying. The 1-butanol developer after shaking with pH 5.6 acetate buffer has a much higher ratio of acetic acid to acetate than does the original buffer solution.

The improvement in tobacco alkaloid chromatograms due to pretreatment of the sheet with pH 5.6 buffer is not considered to be solely due to pH control. Chromatograms have been run on sheets pretreated by spraying with a 1% aqueous solution of sodium chloride and using as developer 1-butanol saturated with 1% sodium chloride solution. R_f values were similar to those reported here. Symmetry of some spots was essentially as good as that on sheets sprayed with pH 5.6 acetate buffer. Other salts have given good chromatograms. The effectiveness of sodium chloride suggests that improvement may be expected, in some cases, by increasing the ionic concentration of the nonmobile phase. The use of either neutral or buffer salts on the paper may be regarded as increasing the disparity between the mobile and nonmobile phase, and contributing to the stability of each phase and the immiscibility of the system.

In a variation of the standard method chromatograms can be run for 1 to 2 hours on small sheets using a 1-quart unlined Mason jar as the chamber. In this technique the R_f's are different from those in the standard method, these differences being enough to change the order of some solutes. This short method can be very useful if one is interested only in the major components of samples.

Suitable extracts have been obtained from tobacco samples with alcohol or acetone. The sample solution must not have a low pH when applied to paper. It is highly satisfactory to use fairly neutral samples dissolved in volatile organic solvents, such as ether, acetone, chloroform, or alcohol. Frequently, it is expedient to evaporate the original solvent in the presence of hydrochloric acid. The alkaloid hydrochlorides can then be taken up in a small amount of pH 5.6 buffer, or a mixture of buffer and ethyl alcohol.

Generally in paper chromatography by the ascending method the prepared paper is formed into an open-end cylinder and allowed to stand in the developer. It is helpful to sew the edges with No. 8 white cotton thread in such a way that they are held close together but not touching.

A number of detection reagents may be used for tobacco alkaloids and related materials. They are not equally sensitive. Some are useful for special applications. Reagents which should be mentioned are iodine vapor, iodoplatinic acid, and diazotized sulfanilic acid. The latter is useful in detecting the pyrrole ring. A *p*-aminobenzoic acid spray, followed by exposure of the sheet to cyanogen bromide vapors after drying appears best suited for general use. Other aromatic amines such as aniline, 2-naphthylamine, and benzidine have been substituted for *p*-aminobenzoic acid. The use of crystalline cyanogen bromide, rather than a saturated aqueous solution is recommended. (Care must be exercised in the use of the cyanogen bromide and amines used to detect the pyridine ring in the Kōnig reaction, because of their toxicity.)

Using *p*-aminobenzoic acid, colored spots, shades of yellow and red, begin to appear immediately on exposure, with maximum development in 10 to 15 minutes. It is well to encircle colored spots with a soft pencil, because weaker spots disappear in an hour or two. The color of an individual spot is of doubtful use in its identification. With many alkaloids reddish colors are favored by high concentration and low pH, while yellow colors are favored by the opposite conditions.

The chromatographic method presented is adaptable to the identification and estimation of the various alkaloids present in varieties and grades of tobacco available to the manufacturer. It should also be useful in following the changes that take place in these substances when they are vaporized and carried in a smoke stream.

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Paper Chromatographic Study of Metal Beta-Diketone Chelates

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In a continuation of the study of the utilization of metal β-diketone chelates in the paper chromatographic separation of ionic mixtures, 2-thenoylperfluorobutyrylmethane and 2-furoylperfluorobutyrylmethane were investigated. Separation of iron(III), copper(II), nickel(II), cobalt(II), and manganese(II) chelates was effected by the use of a petroleum ether B-methanol mixture. The potentialities of the two chelating agents as a spot test reagent were investigated. Several factors affecting the chromatographic separation of the mixture of metal chelates were studied qualitatively. While no one factor could be found to explain the relative sequence of R_f values, a consideration of solubility and total polarization of the various metal chelates gave the proper R_f sequence.

THIS paper is a continuation of the study of the utilization of β-diketone chelates for the chromatographic separation of ionic mixtures (*2–4*). 2-Thenoylperfluorobutyrylmethane and 2-furoylperfluorobutyrylmethane are relatively new β-diketones which were first prepared by Barkley and Levine in 1951 (*1*). No references to their uses were found in the literature.

The study of these chelating agents may be divided into three parts: (1) an investigation of their potentialities as analytical reagents for spot tests; (2) the paper chromatographic separation of five common metals as the chelates; and (3) a qualitative interpretation of the relationship among R_f values, solubility, and adsorption affinity of the different metal chelates.

It is of interest to study some of the factors involved in the chromatographic separation of the chelates. As the authors have noted before in the case of other metal β-diketone chelates (*3, 4*), the relative R_f sequence can be explained qualitatively by comparison of the solubility of the metal chelates in the developing solvent with the relative polarizations of the metal chelates. It is assumed in this discussion that solubility is the driving force up the paper and that the adsorption affinity of the metal chelate for the paper is chiefly responsible for the holding force. These two processes are in constant competition and are here believed to govern the separations.

REAGENTS AND APPARATUS

2-Furoylperfluorobutyrylmethane (Mid-Continent Chemical Corp.).

2-Thenoylperfluorobutyrylmethane (Mid-Continent Chemical Corp.).

Petroleum ether B (bulk grade) saturated with anhydrous sodium sulfate.

Methanol, C.P., saturated with anhydrous sodium sulfate.

Sodium sulfate, C.P., anhydrous.

Alcoholic solution of dithiooxamide, 0.03*M*.

Aqueous solution of sodium hydroxide, 0.05*M*.

Benzidine solution, 0.05 gram of benzidine base dissolved in 10 ml. of acetic acid, diluted to 100 ml. with water, and filtered.

Ethyl acetate, C.P.

Dioxane (technical grade) purified by refluxing with 0.1*N* hydrochloric acid, separated by addition of excess concentrated sodium hydroxide, dried over potassium hydroxide, and distilled over sodium wire.

Beckman Model DU spectrophotometer.

B-S differential refractometer (Phoenix Precision Instrument Co.).

Bausch & Lomb grating monochromator with tungsten lamp.

Sargent Model V chemical oscillometer with oscillometric cell, compensator, and cell holder, Type A.

PROCEDURE

Spot test procedures were applied to most of the common metal ions in determining the usefulness of 2-thenoylperfluorobutyrylmethane and 2-furoylperfluorobutyrylmethane as chelating agents. One drop of 0.1% ionic solution was placed in a spot plate and 1 drop of 5% sodium acetate was added, followed by 1 drop of 10% chelating agent in ethyl alcohol. When good tests were obtained, the ionic solutions were diluted and the tests were repeated to determine the limit of sensitivity. All tests were made with only 3 drops or about 0.1 ml. of solution.

Five of the more common ions that gave good spot tests were selected to be studied for chromatographic separations of metal chelates. The pure metal chelates were formed by addition of a 10% ethanolic solution of the chelating agent to aqueous solutions of the metal ions buffered to a pH of approximately 7 with sodium acetate. The metal chelates precipitated readily and were filtered and dried. Each of the metal chelates, except that of iron(III), was dissolved in a small amount of hot ethyl alcohol, reprecipitated by addition of water, filtered, and dried over calcium chloride in a vacuum desiccator for several days. The iron(III) chelate was treated with boiling water and formed a heavy oily liquid. The water was removed and the oily layer, which hardened upon cooling, was pulverized and dried over calcium chloride in a vacuum desiccator for several days. One tenth of a gram of each metal chelate was dissolved in 10 ml. of ethyl alcohol, giving a concentration of 10 grams per liter. Approximately 5 μl. of each solution were used in spotting the paper for the analysis. Fifty micrograms of each metal chelate or less than 5 γ of each metal were present in the final spot.

Hydrometer cylinders, 43 cm. tall and 7 cm. in diameter, served as chromatographic chambers. A part of the cylinder wall was lined with filter paper soaked with the solvent in order to saturate the chamber more efficiently. Twelve hours were then allowed for complete saturation of the chamber.

Whatman No. 1 filter paper strips 2.5 inches wide were spotted with solutions of the metal chelates and dried in air for 1 hour. The strips were then placed in the chamber, saturated with vapor, and equilibrated for 1 hour before immersion in the solvent. The chromatograms were developed completely in 2 hours, the solvent front having ascended approximately 27 cm.

The position of each of the metal chelates in the developed chromatogram was determined in the following manner. The position of the iron(III) chelate was easily detected by its visible yellow color. Although the iron(III) chelate is red in solution, it appears yellow on paper when very dilute and diffuse. The cobalt(II), nickel(II), and copper(II) chelates gave yellow, blue, and gray-black colors, respectively, when sprayed with an alcoholic solution of dithiooxamide. The manganese(II) chelate was determined by spraying the paper first with 0.05*N* sodium hydroxide and then with benzidine reagent to give a green color.

Neither the dithiooxamide reagent nor the benzidine reagent was sensitive in the presence of the other. Therefore, each paper was spotted in two different places about 1 inch apart and approximately 1 inch from the bottom of the paper. After the chromatogram was developed, it was cut into two strips, one

being used for the dithiooxamide test and the other for the benzidine test.

Equal molar mixtures of the ions were converted into mixtures of the chelates by precipitating the chelates in the same manner used for the preparation of pure chelates. The chelate mixture was then extracted with ethyl acetate. The ethyl acetate solution of the mixed chelates was then used to spot the paper instead of solutions of the individual pure chelates. Similar chromatograms were obtained regardless of whether the chelates were prepared and extracted together or mixed together as one spot on the chromatographic paper.

To verify that the metal chelates were being chromatographed and not the ions, a paper was spotted with the metal nitrates and chromatographed under conditions identical to the procedure used with the metal chelates. No migration of ions was observed.

Preliminary work indicated that petroleum ether and methanol would make a desirable solvent mixture. Petroleum ether alone moved the iron(III) chelate near the solvent front and the copper(II) chelate about half way. The nickel(II), cobalt(II), and manganese(II) chelates did not move. Methanol moved all the metal chelates near the solvent front, except the manganese(II) chelate, which trailed behind the solvent front. A number of other polar solvents were tried mixed with petroleum ether, but none of the mixtures gave good separations.

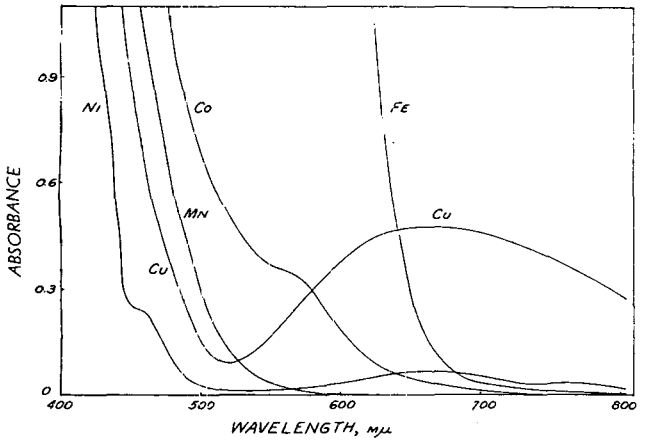

Figure 1. Absorption spectra for 0.01*M* solutions of metal chelates in ethyl acetate

Small quantities of methanol mixed readily with petroleum ether B, but upon standing two layers were noticed. It was observed that this immiscibility was due to moisture absorbed from the air. The addition of anhydrous sodium sulfate to the petroleum ether–methanol mixture eliminated this difficulty.

A Sargent Model V chemical oscillometer with a fixed frequency of 5×10^6 cycles per second was used for the relative dielectric constant measurements of standard solutions of the metal chelates in 1,4-dioxane and in ethyl acetate.

Absorption spectra of 0.01*M* solutions of the metal chelates in ethyl acetate were obtained using a Beckman DU spectrophotometer. Refractive index difference measurements between pure ethyl acetate and 0.01, 0.02, and 0.04*M* solutions of the metal chelates in ethyl acetate were obtained using a B-S differential refractometer with a Bausch & Lomb grating monochromator as the light source.

Solubility measurements were obtained by evaporating an aliquot of a saturated solution of the metal chelate to dryness in vacuo and weighing the residue. Saturated solutions of the metal chelates in various mixtures of petroleum ether and methanol were periodically shaken for 1 hour, and centrifuged. Anhydrous sodium sulfate was added in small quantities to remove any moisture absorbed from the atmosphere. An aliquot of the saturated solution was withdrawn and transferred to a small preweighed

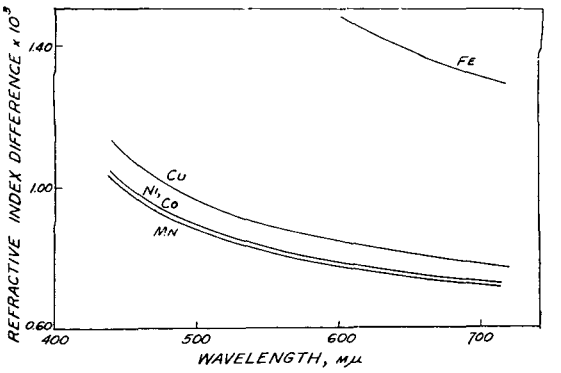

Figure 2. Refractive index differences between pure solvent and 0.01*M* solutions of metal chelates in ethyl acetate versus wave length

evaporating dish. The solvent was removed by evaporation in a vacuum desiccator and the residue was determined by difference.

RESULTS

2-Thenoylperfluorobutyrylmethane. 2-Thenoylperfluorobutyrylmethane is an excellent chelating agent for a large number of metal ions. Table I gives the colors and sensitivities of the test in 0.1 ml. of solution.

Mercury(II), magnesium, zinc, barium, strontium(II), and calcium formed white precipitates. Rhodium(III), iridium(IV), platinum(IV), tin(IV), tin(II), antimony(III), bismuth(III), mercury(I), chromium(III), cadmium(II), lanthanum(III), aluminum, lithium, and tungstate did not give colored solutions or precipitates with 2-thenoylperfluorobutyrylmethane.

The R_f values shown in Table II are average values obtained from several chromatograms. The iron(III) and copper(II) chelates can be separated completely from each other and from the other metal chelates in pure petroleum ether B. The most efficient separations were obtained with 92% petroleum ether B and 8% methanol. The nickel(II) and cobalt(II) chelates were not completely resolved, but were sufficiently resolved to give reliable tests for each.

Table I. Colors and Sensitivities of 2-Thenoylperfluorobutyrylmethane Spot Tests with Various Metal Ions

Ion	Color	Sensitivity, γ
Fe^{++}	Purple	0.3
Fe^{+++}	Red	2
Co^{++}	Orange	2
Mn^{++}	Yellow	2
Ce^{++++}	Brown	2
V^{+++}	Brown	2
UO_2^{++}	Yellow	2
Au^{+++}	Black	2
Cu^{++}	Green	5
Ni^{++}	Green	5
Pd^{++}	Yellow	5
Cu^{+}	Green	10
Ti^{++++}	Yellow	25
Tl^{++++}	Yellow	75
Ag^{+}	Yellow	150
Pb^{++}	Yellow	500
MoO_4^{--}	Yellow	500

Table II. R_f Values of Various Metal 2-Thenoylperfluorobutyrylmethane Chelates in Mixed Solvents of Petroleum Ether B and Methanol

Petroleum Ether, %	Methanol, %	R_f Values of Metal Chelates				
		Fe	Cu	Ni	Co	Mn
100	0	0.95	0.48	0.00	0.00	0.00
97	3	0.96	0.92	0.67	0.59	0.23
94	6	0.89	0.83	0.57	0.52	0.32
92	8	0.82	0.77	0.51	0.47	0.35
90	10	0.81	0.76	0.50	0.47	0.36

Table III. Solubility of Metal 2-Thenoylperfluorobutyrylmethane Chelates in Mixed Developing Solvents of Petroleum Ether B and Methanol

Petroleum Ether, %	Methanol, %	Solubilities of Metal Chelates, Mg./Ml. Fe	Cu	Ni	Co	Mn
100	0	14.4	0.7	0.0	0.0	0.0
97	3	56.6	7.6	5.2	4.9	6.2
94	6	96.3	12.8	13.4	11.3	12.2
91	9	..	20.4	22.5	19.7	22.3

Table IV. Capacitance Measurements of Solutions of Metal Chelates in Dioxane and Ethyl Acetate

Chelates	Dioxane, 0.01*M*	Ethyl Acetate, 0.01*M*
Iron	14,551	18,382
Manganese	14,419	18,415
Cobalt	14,416	18,358
Nickel	14,411	18,360
Copper	14,371	18,327
Solvent	14,191	18,190

Table V. Colors and Sensitivities of Spot Tests for Common Metal Ions Using 2-Furoylperfluorobutyrylmethane

Ion	Color	Sensitivity
Fe^{++}	Purple	0–3
Fe^{+++}	Red	2
Co^{++}	Orange	2
Mn^{++}	Yellow	2
UO_2^{++}	Yellow	2
V^{+++}	Red	2
Au^{+++}	Black	2
Ce^{++++}	Brown	2
Cu^{++}	Green	5
Ni^{++}	Green	5
Pd^{++}	Yellow	5
Cu^{+}	Green	15
MoO_4^{--}	Orange	15
Cr^{+++}	Brown	25
Ti^{++++}	Yellow	50
Ag^{+}	Yellow	500
Ti^{+}	Yellow	500
WO_4^{--}	Tan	500

Relative capacitance measurements of solutions of the metal chelates in dioxane and in ethyl acetate are given in Table IV.

At a wave length of 700 mμ only the solution of the copper(II) chelate absorbs appreciably (Figure 1).

Refractive index differences between pure solvent and 0.01*M* solutions of the metal chelates in ethyl acetate *vs.* wave length are given in Figure 2. Each of the metal chelate solutions gives a normal dispersion curve.

Refractive index differences between pure solvent and various concentrations of the metal chelates in ethyl acetate are given for a wave length of 700 mμ in Figure 3.

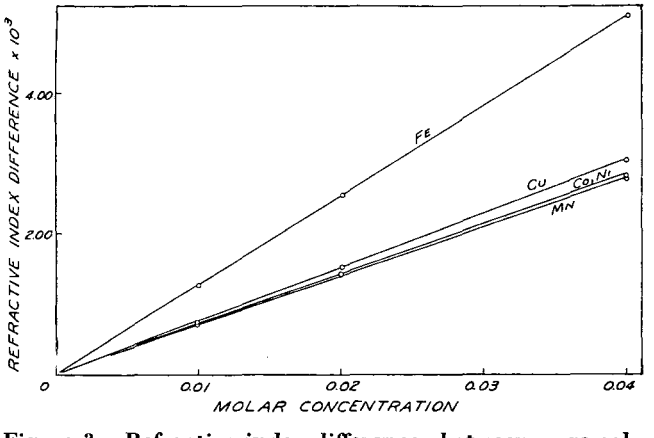

Figure 3. Refractive index differences between pure solvent and various concentrations of metal chelates in ethyl acetate at a wave length of 700 mμ

2-Furoylperfluorobutyrylmethane. The potentialities of 2-furoylperfluorobutyrylmethane as a spot test reagent are outlined in Table V.

Lanthanium(I), magnesium, calcium, strontium(II), barium, zinc, cadmium, mercury(II), aluminum, and zirconium(IV) gave white precipitates.

Lithium, mercury(I), lead(II), tin(II), antimony(III), bismuth(III), rhodium(III), platinum(IV), iridium(IV), tin(IV), dichromate and chromate ions do not produce colors or precipitates with the chelating agent under the conditions of the above tests.

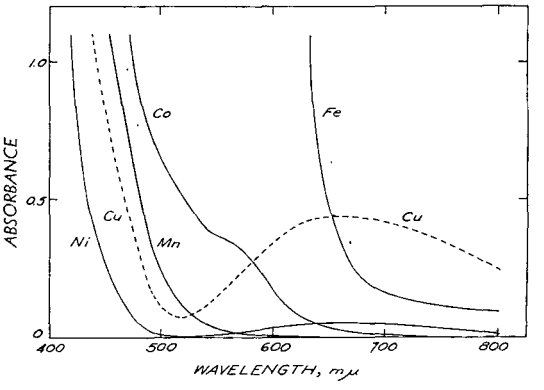

Figure 4. Absorption spectra for 0.01*M* solutions of metal 2-furoylperfluorobutyrylmethane chelates in ethyl acetate

Sharper zones were observed when a small quantity of dioxane was added to the petroleum ether–methanol mixtures.

Absorption spectra of 0.01*M* solutions of the five metal chelates in ethyl acetate are given in Figure 4.

The solubility of the different metal chelates in the developing solvent of 92% petroleum ether B, 7% methanol, and 1% dioxane is given in Table VII.

Table VI. R_f Values for Metal 2-Furoylperfluorobutyrylmethane Chelates in a Mixture of 92% Petroleum Ether B, 7% Methanol, and 1% Dioxane

Metal Chelate	R_f Value
Iron(III)	0.84
Copper(II)	0.77
Nickel(II)	0.50
Cobalt(II)	0.46
Manganese(II)	0.20

Table VII. Solubility of Metal 2-Furoylperfluorobutyrylmethane Chelates in Developing Solvent

Metal Chelate	Solubility, Grams/Liter
Copper(II)	55.3
Iron(III)	32.1
Nickel(II)	19.7
Cobalt(II)	19.0
Manganese(II)	18.2

Table VIII. Relative Capacitance of 0.025*M* Solutions of Metal 2-Furoylperfluorobutyrylmethane Chelates in Ethyl Acetate

Metal Chelate	Relative Capacitance
Manganese(II)	19,840
Iron(III)	19,820
Nickel(II)	19,788
Cobalt(II)	19,779
Copper(II)	19,591
Solvent	19,244

The difference in refractive index between pure ethyl acetate and 0.01*M* solutions of the five metal chelates in ethyl acetate are plotted at different wave lengths in Figure 5. All of the metal chelate solutions gave normal dispersion curves. A flat portion of the dispersion curves shown in Figure 5 occurs around 700 mμ. The differences in refractive index between pure ethyl acetate and varying molar concentrations of the metal chelates in ethyl acetate are plotted for a wave length of 700 mμ in Figure 6. All the metal chelate solutions give straight-line relationships.

DISCUSSION

A measure of the relative dielectric constants of dilute equimolar solutions of similar solutes in an ideal solvent indicates the relative total polarization of the solute molecules. The determination of relative dielectric constants usually involves the measurement of the capacitance of a cell filled first with solution and then with the solvent. The capacitance of the cell is measured at a frequency of approximately 10^6 cycles per second because the time of relaxation of the molecules is short in comparison with the period of alternation.

Because interactions occur between solute and solvent, the polarization measured with the high frequency oscillometer is only an approximation of the total polarization.

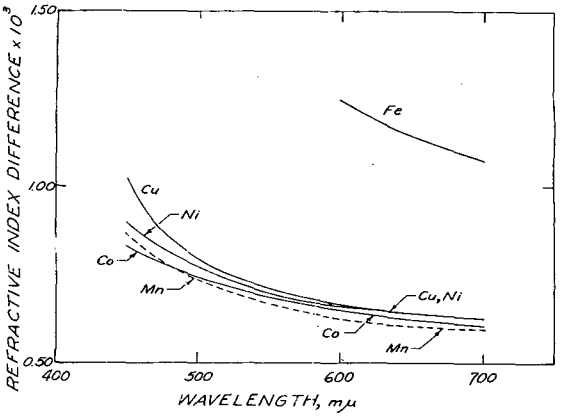

Figure 5. Refractive index difference between pure solvent and 0.01*M* solutions of metal 2-furoylperfluorobutyrylmethane chelates in ethyl acetate *vs.* wave length

From the data in Table IV the relative polarizations of the metal chelates in dioxane are iron>manganese, cobalt, nickel> copper; in ethyl acetate they are manganese>iron>nickel, cobalt>copper so that one can conclude the following polarization sequence: iron, manganese>nickel, cobalt>copper.

Generally the greater the polarization or polarizability of a substance, the more strongly will it be adsorbed. For this reason, polarization should give a measure of the relative adsorption affinities of the metal chelates for the paper. Paper contains many hydroxyl groups and these tend to attract a polar substance more strongly than a nonpolar one.

A qualitative explanation of the observed R_f sequence for the 2-thenoylperfluorobutyrylmethane chelates may be seen from the following. Consideration of the adsorption affinity data leads one to expect the R_f sequence copper>nickel, cobalt>iron, manganese, whereas the solubility data suggest the sequence iron>nickel, copper, manganese, cobalt. From the solubility data alone, one would predict that the R_f value of the iron(III) chelate would be large in comparison with the other R_f values, which would be similar. In this system the separation of the other four chelates must depend on different adsorption affinities. The solubility of the iron(III) chelate apparently is great enough to overcome the high adsorption affinity. Since the solubility

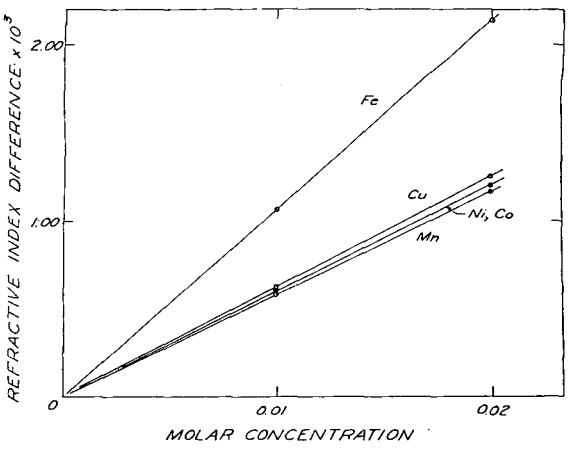

Figure 6. Refractive index difference between pure solvent and various concentrations of metal 2-furoylperfluorobutyrylmethane chelates in ethyl acetate at a wave length of 700 mμ

and adsorption affinity of the nickel(II) and cobalt(II) chelates are similar, it might be expected that this solvent system cannot completely separate them. Actually, the nickel chelate is slightly more soluble than the cobalt chelate and this may account for its partial separation for cobalt. Therefore, a qualitative explanation for the R_f sequence iron>copper>nickel>cobalt> manganese is possible from solubility and capacitance measurements.

A first-order approximation of the polarizability (electron polarization) of solute molecules may be obtained from the refractive index difference between a solution and its solvent, provided the measurements are made at a wave length removed from the region of absorption (*3*). Debye and Nauman (*5*) have used the equation $\mu - \mu_0 = n\alpha$ in light scattering work. The terms μ and μ_0 are the refractive indices of the solution and solvent, respectively, n is the number of particles per cubic centimeter of solvent, and α is the polarizability of the solute molecule.

Anomalous behavior is observed when the refractive index measurements are made at a wave length within a region of absorption. A wave length corresponding to the flat portion of the dispersion curves of the metal chelates given in Figure 2 was taken for the measurements of refractive index difference given in Figure 3. For equal molar solutions the refractive index difference, $\mu - \mu_0$, is directly proportional to the polarity, α. On the basis of polarizability the R_f sequence would be nickel, cobalt, manganese>copper>iron and when compared with the solubility data would predict and R_f sequence iron>nickel, cobalt, manganese>copper. This is not the proper R_f sequence and no correlation is noted between R_f value and polarizability. Other authors have noted this lack of correlation between amount of absorption and polarizability.

A similar treatment of the data collected for the 2-furoylperfluorobutyryl-methane chelates will lead one to a qualitative explanation of the observed R_f sequence.

The obvious exception to such an explanation is the iron(III) chelate. However, one should recognize the great structural dissimilarity between the iron(III) chelate and the other metal chelates studied.

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Conductometric Method for Rapid Chemical Analysis of the Nitric Acid–Nitrogen Dioxide–Water System

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A method employing the measurement of the specific electrolytic conductance of a pair of duplicate samples of fuming nitric acid permits a rapid, fairly accurate determination of nitric acid, nitrogen dioxide, and water without requiring gravimetric or volumetric measurements. At a given temperature the conductance of a sample of fuming nitric acid, together with the conductance of a duplicate sample saturated with anhydrous potassium nitrate, gives sufficient information to determine the composition of the sample. Values of the specific conductance of saturated solutions of potassium nitrate in fuming nitric acid were obtained at 0° C. for various fuming nitric acid mixtures, and the resulting data necessary for utilizing this conductometric method of analysis in the composition ranges 0 to 12 weight % nitrogen dioxide, 0 to 10 weight % water, 12 to 20 weight % nitrogen dioxide, and 0 to 6 weight % water are presented in graphical and tabular form. By this method nitrogen dioxide can be determined on an absolute basis to ±0.3 weight % and water to ±0.3 weight % in the composition range studied. The underlying principles of this method, together with the chemistry of nitric acid solutions, are discussed.

IT IS desirable to have a simple, rapid method for the determination of the composition of samples of fuming nitric acid [the three-component system nitric acid–nitrogen dioxide–water for all compositions in nitric acid of about 70 weight % or greater] in terms of nitric acid, nitrogen dioxide, and water. Throughout this report compositions are referred to on the basis of formal weight percentage for each compound—i.e., in terms of the formula of the compound disregarding the molecular species that may result from its solution. A summary of several wet methods of analysis of fuming nitric acid can be found in the literature (*16*). Precise techniques for volumetric methods of analysis of fuming nitric acid containing 0 to 2.5% nitrogen dioxide and 0 to 5% water have recently been developed (*3*). Such methods of analysis, however, are time-consuming and require highly trained personnel. Also the species water is determined by difference, so that impurities in the fuming nitric acid may result in large errors in determining water in systems containing only a few per cent of water.

Based on the fact that some physicochemical properties of fuming nitric acid are markedly a function of composition, several instrumental methods of analysis have been devised (*17*). To define unambiguously the composition of a three-component system, it is necessary to determine the percentage of only two of the components by means of at least a pair of separate physical measurements. To be most satisfactory, each of the pair of measured physical properties should be markedly affected by only one of the components of the system.

Spectrophotometric measurements at suitable wave lengths in the infrared range, where water strongly absorbs (*18*), and in the visible range, where nitrogen dioxide strongly absorbs (*13*), have been suggested as analytical tools. It is also possible that spectrophotometric measurements at suitable wave lengths in the ultraviolet range, where nitrogen pentoxide and nitrate strongly absorb (*12*), would similarly provide a convenient instrumental method of analysis. A pair of measurements of absorbance on an unknown sample at suitable wave lengths in two different spectral ranges or at one suitable wave length, together with the use of dilution techniques (*13*), gives sufficient information to establish the composition of the system for most ranges of composition encountered in commercial samples of fuming nitric acid. For general use, however, the spectrophotometric method has the disadvantage of requiring somewhat bulky and delicate instrumentation. Based on the fact that fuming nitric acid solutions are ionized, another instrumental method involving measurement of conductance might be suitable for analyses.

THEORY OF CONDUCTOMETRIC MEASUREMENTS

Ionic Equilibria in Fuming Nitric Acid. Various physical properties of liquid fuming nitric acid indicate that ionic equilibria prevail in solution. Measurements of the Raman spectra (*8*) indicate that liquid nitric acid undergoes self-ionization to give nitronium ions (NO^+_2) and nitrate (NO^-_3) ions according to the equilibrium expression

$$2HNO_3 \rightleftharpoons NO_2^+ + NO_3^- + H_2O \qquad (1)$$

Measurements of freezing points (*5*) and of specific conductance (*14, 15*) of nitric acid solutions support the existence of this ionization.

In nitric acid solutions containing over 3% water at 25° C., ionization to hydronium (H_3O^+) and nitrate ions according to the following equation becomes prominent (*5, 14, 15*):

$$HNO_3 + H_2O \rightleftharpoons H_3O^+ + NO_3^- \qquad (2)$$

In solutions of nitrogen dioxide in nitric acid, nitrosonium (NO^+) and nitrate ions have been identified by Raman spectral studies (*7*), and the existence of these species is supported by conductance measurements (*14, 15*).

The resulting ionic equilibrium may be written

$$2NO_2 \rightleftharpoons N_2O_4 \rightleftharpoons NO^+ + NO_3^- \qquad (3)$$

A convenient conductometric method of analysis employing saturated solutions of anhydrous potassium nitrate is discussed below; therefore the chemistry of these solutions should be mentioned. Raman spectral studies have shown that the following equilibrium involving association of nitric acid molecules with nitrate ions exists (*1*):

$$2HNO_3 + KNO_3 \rightleftharpoons K^+ + (HNO_3)_2\,NO_3^- \qquad (4)$$

The effect of this equilibrium on conductance of solutions of potassium nitrate in nitric acid is discussed under results.

General Conductometric Methods of Analysis. The specific conductance of an ionized liquid is an intensive property which is indicative of the number, charge, and mobility of positive and negative ions present. The number of ions depends on extent of ionization of the molecular species in solution, and the mobility of the resulting ions depends on the nature of their geometry as well as on the viscosity and temperature of the solution. The specific conductance of these ionic solutions can be determined by measuring the resistance of a column of solution of known dimensions between two electrodes. The specific conductance, κ, which is measured in ohms per centimeter, is then related to the total measured resistance R (ohms) of the solution of cross-sec-

tional area a (square centimeters) and length l (centimeters) by the following equation:

$$\kappa = \frac{l}{aR} \qquad (5)$$

Usually the dimensions of the electrical path of a conductance cell are not directly measured but are determined by measuring the resistance of a solution whose specific conductance has been measured earlier in a cell of precisely known dimensions. The unknown ratio of l/a, the so-called cell constant, is thus obtained for a given cell (*19*). After the cell constant is determined, the specific conductance of a solution may be obtained by measuring its resistance and applying Equation 5. If conductance is a marked function of composition it may be used as a convenient method of chemical analysis (*4*).

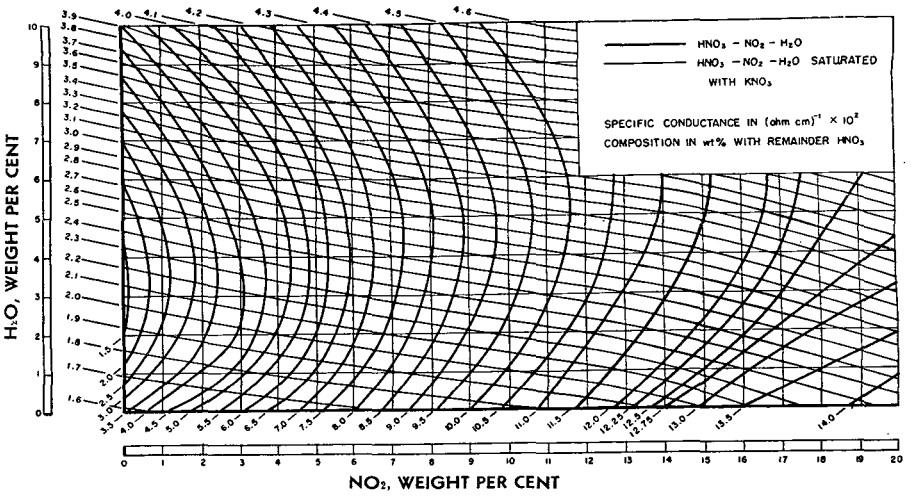

Figure 1. Curves of constant specific conductance for nitric acid solutions at 0° C.

Conductometric Methods of Analysis of Fuming Nitric Acid. The specific electrical conductance of the nitric acid–nitrogen dioxide–water system is markedly a function of composition, as is shown in Figure 1, in which each heavy solid curve represents the locus of all compositions having a constant specific conductance at 0° C. (*14*). To establish definitely the composition of a sample, it is then necessary to measure an additional physical property whose curves of constant value intersect the heavy solid conductance curves of Figure 1. One suggested method (*13*) employs a measurement of optical absorbance in addition to that of conductance, but this procedure is rather elaborate, requiring two different instruments. A method employing only one type of measurement such as electrical conductivity has practical advantages.

It is possible that, by adding or removing a constant amount of one of the components of the system (or adding substances that affect the conductance), the resulting conductance, together with the initial conductance, will determine the composition of the system. An analytical method coupling conductometric measurements with dilution techniques has been applied successfully to a narrow range of compositions in the nitric acid–rich region and requires precise additions of known amounts of water to the sample (*2*). Since water is directly or indirectly involved in the ionic equilibria represented by Equations 1 through 3, it is evident that this species should alter the conductance. Such behavior is borne out by the solid curves of Figure 1. Although conductometric methods in general are precise and employ rugged, portable, and relatively inexpensive equipment (*9*), a rapid routine method of analysis utilizing this type of measurement is desired which will not in addition require gravimetric or volumetric measurements. A procedure eliminating gravimetric or volumetric manipulations could be used by personnel having little chemical training.

Several procedures employing a pair of conductometric measurements and eliminating gravimetric or volumetric techniques were tested. One procedure involved removing the nitrogen dioxide from fuming nitric acid by bubbling dry air through the solution and measuring the resultant change in conductance. This method proved too tedious to be of use as a rapid, simple method of analysis. Another procedure employed solid drying agents in an attempt to remove water and thus to measure a reproducible change in conductance, but no suitable drying agent was found. A further method involved saturating the solution with a solid compound which would measurably alter the conductance of the system. The use of various solid nitrates as additives is suggested by the fact that nitrate ion is involved in the ionic equilibria represented by Equations 1 through 4 and should therefore affect the conductance in a fashion dependent on initial composition.

A procedure has been developed employing saturated solutions of anhydrous potassium nitrate. The conductance of an unknown sample of fuming nitric acid is measured at a given temperature, and the composition is established as lying on one of the heavy solid curves in a diagram such as Figure 1. A duplicate sample is saturated with anhydrous potassium nitrate, and the conductance is measured at the same temperature. The ice point was chosen as standard temperature in the present investigation. An excess amount of the anhydrous potassium nitrate solid phase is present during the conductance measurement to ensure that the liquid-phase composition is at its saturation point. From the nature of phase equilibria it is evident that the state of such a heterogeneous system at equilibrium is independent of the relative amount of liquid and solid present. Herein lies the principle which enables one to measure the electrical conductance of fuming nitric acid solutions saturated with anhydrous potassium nitrate without having to measure directly the amounts of solid potassium nitrate or solution present. In Figure 1 each light curve represents the locus of all compositions of saturated potassium nitrate solutions with constant conductance of 0° C. It is evident from the nature of the two intersecting sets of conductance curves that

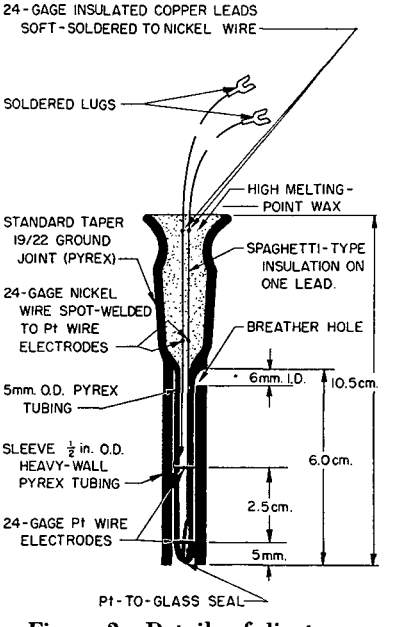

Figure 2. Details of dip-type conductance cell

the composition of the system may be defined from this pair of measurements. Throughout this report, original composition of fuming nitric acid before addition of potassium nitrate is given.

EXPERIMENTAL PROCEDURES

Equipment for Conductometric Measurements. A small dip-type glass cell with platinum electrodes was designed for the special conditions of the conductometric measurements. The details and dimensions of the cell used are shown in Figure 2. (Cells to these specifications are manufactured by R. E. Logan, technical glassblowers, 1342 East Wilson Ave., Glendale 6, Calif.) The ring-shaped pair of platinum electrodes is made from 24-gage platinum wire wrapped once circumferentially around a slight indentation in the borosilicate glass tubing 5 mm. in outer diameter. The ring is kept from springing open by spot welding the end of the wire to the ring. The other ends of both the platinum wires are sealed through the 5-mm. (outside diameter) borosilicate glass tubing and is spot-welded to a short length of 24-gage nickel wire, which in turn is soft-soldered to copper leads. In a recent modification a tungsten-to-glass seal was used, one end of the 24-gage tungsten wire being welded to the platinum electrode and the other end to the nickel lead. This design consistently gave a more leakproof seal.

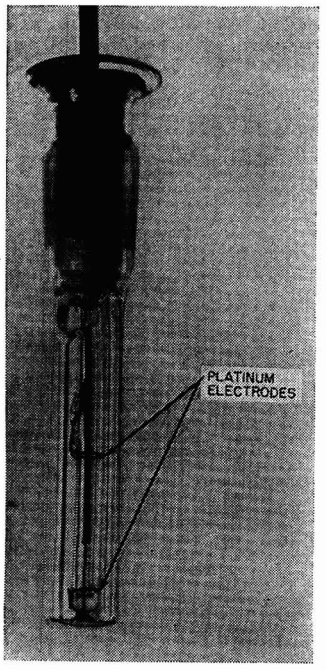

Figure 3. Conductance cell

One of the wires which extend up the interior of the 5-mm. tubing is insulated with electrical spaghetti. This general design prevents contact of the leads with fuming nitric acid. To provide some rigidity to the leads, a wax of high melting point is poured in the top of the 19/22 standard-taper glass joint. Making the glass-to-platinum seal is facilitated by hammering the platinum thin at the point of juncture. A glass sleeve constructed of heavy-walled borosilicate glass tubing 0.5 inch in outer diameter surrounds the electrodes in such a manner as to give an electrical path of constant dimensions (Figure 2). A hole 6 mm. in diameter near the top of this sleeve prevents air from being trapped in the cell when it is dipped into the sample of fuming nitric acid. The electrodes are platinized by immersing them in a solution containing 3 grams of platinic chloride and 0.02 gram of lead acetate dissolved in 100 ml. of distilled water and by putting 6-volt direct current across the terminals. Intermittently the polarity of this voltage is reversed (*10*).

As receptacles for the fuming nitric acid samples during the conductance measurements, borosilicate glass bottles (Corning No. 1500) of 125-ml. capacity with 19/22 standard-taper glass stoppers were found to be convenient. The mating joint on the dip cell fits into the standard-tapered neck of the bottles. The fuming nitric acid sample was contained in the bottle with its glass stopper until the conductance measurement was made, at which time the electrode assembly replaced the stopper. In this manner excessive exposure to moist air and loss of volatile components from the system were avoided. The conductance cell is shown in Figure 3 and the assembled equipment in Figure 4. In Figure 4 a solid phase of anhydrous potassium nitrate which was used in the method described later can be seen in the bottom of the bottle.

For the conductance measurements a commercial Type RC conductivity bridge (Industrial Instruments, Inc.) was employed (*9, 10*). In principle the circuit consists of an alternating current Wheatstone bridge with the conductivity cell and liquid sample comprising one leg of the bridge. The resistance of the solution is determined by obtaining a balance on focusing an electron-ray "eye tube" null indicator. To reduce effects due to polarization,

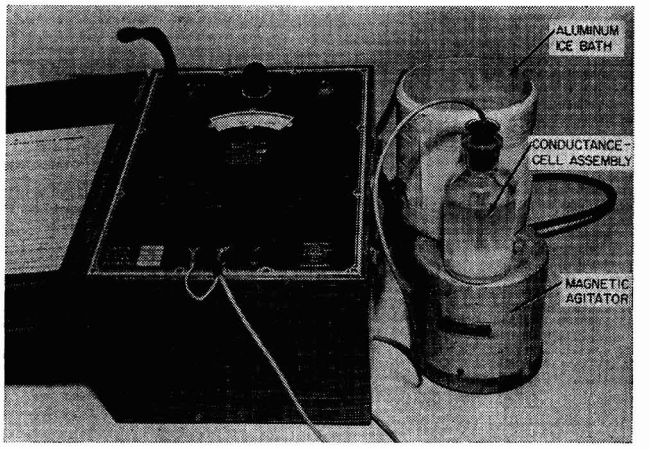

Figure 4. Equipment for measuring conductance of nitric acid solutions

the bridge is operated with alternating current at 1000 cycles per second. The cell in position in the bottles as shown in Figure 4 was calibrated at 0° C. with standard 1.0*N* potassium chloride solutions whose specific conductances are precisely given in the literature (*11, 19*). The cell having the dimensions shown in Figure 2 and used throughout most of the measurements had a constant of about 10 $cm.^{-1}$ With this type of cell, values of resistance from about 70 to 670 ohms were encountered for fuming nitric acid solutions in the composition range studied. Sharp balance was obtained on the conductance bridge, as evidently in this range the resistances were low enough to prevent insulation leakage and yet high enough to prevent polarization due to excessive current densities. Although the commercial type of bridge used did not give the maximum attainable precision in the measurement of conductance, the irreproducibility due to chemical factors was limiting, so that the inexpensive commercial bridge appeared to be suitable.

Materials. Nitric acid was prepared by vacuum distillation at about 40° C. from a mixture of Baker and Adamson reagent grade potassium nitrate and reagent grade (95%) sulfuric acid by the procedure described in the literature (*6*). Nitrogen dioxide (obtained from the Allied Chemical and Dye Corp.) was purified by crystallization. Laboratory-prepared distilled water was used. Dried Baker and Adamson reagent grade potassium nitrate was added to samples of fuming nitric acid as described later. It is imperative that the potassium nitrate contain no nitric acid as an impurity, for the presence of the compound $KNO_3 . 2HNO_3$ vitiates the method described. Samples of fuming nitric acid in the composition range 0 to 20% nitrogen dioxide and 0 to 10% water were prepared by gravimetrically adding nitrogen dioxide and water to pure nitric acid.

Miscellaneous Conductometric Methods of Analysis of Fuming Nitric Acid. By completely removing nitrogen dioxide from a sample, the final conductance together with the initial conductance defines the composition of the acid. Nitrogen dioxide was removed from a sample of fuming nitric acid by bubbling dried air at a volumetric flow rate of about 100 cc. per minute through the sample. A train of two glass scrubbers was used, the first containing pure nitric acid and the second, the unknown acid sample. Both scrubbers were maintained at 50° C., which temperature appears to be high enough to give an elevated vapor pressure of nitrogen dioxide with accompanying rapid evolution of this species and yet low enough to prevent thermal decomposition of nitric acid. Under these conditions at least 20 minutes was required to remove the nitrogen dioxide completely from a sample which initially contained about 15% nitrogen dioxide. The first scrubber in the train had the purpose of heating the air and saturating the effluent gases with nitric acid in order to minimize the amount of nitric acid lost from the second scrubber. The conductance measurements at 0° C. on duplicate samples in which nitrogen dioxide was removed could be reproduced only if previous flow rates and temperatures were very carefully controlled. Accordingly, from a practical standpoint, this method was discarded.

Excess quantities of solid drying agents were added to fuming nitric acid in an attempt to reduce the concentration of water in the liquid phase to a constant value, thus measurably changing the conductance of the solution. Phosphorus pentoxide proved to be too drastic a drying agent, ultimately dehydrating nitric acid to solid nitrogen pentoxide. Calcium sulfate and magnesium

perchlorate were incapable of removing water from liquid nitric acid. Since these methods of removing nitrogen dioxide or water proved impractical, a method employing the addition of solid-phase potassium nitrate to the system was then tested.

Conductometric Method of Analysis of Fuming Nitric Acid Employing Saturated Solutions of Potassium Nitrate. In Figure 1, the intersection of a pair of curves representing conductance measurements at 0° C., one on a sample of fuming nitric acid alone and another on a duplicate sample saturated with potassium nitrate, defines the composition of the system. The heavy lines for fuming nitric acid without potassium nitrate represent data taken from the literature (*14*) and from the present investigation for composition in the range 16 to 20 weight % nitrogen dioxide. The light lines for saturated solutions of potassium nitrate in fuming nitric acid were obtained in the present investigation with the equipment shown in Figure 4. The samples of fuming nitric acid were maintained at 0° C. by immersing the bottles in the aluminum container filled with an ice-water mixture. Approximately 70 grams of dried Baker and Adamson reagent grade potassium nitrate were added through a glass funnel to about 50 ml. of fuming nitric acid at 0° C. in the 125-ml. bottles. The liquid level in the bottle was kept at all times below the breather hole, since the cell was calibrated under this condition. To facilitate the attainment of phase equilibrium between anhydrous potassium nitrate and the fuming nitric acid solutions and to prevent local high temperatures and concentrations due to the heat of solution, the magnetically driven agitator assembly shown in Figure 4 was employed. The potassium nitrate was added at such a rate that a solid phase was always present, and agitation with the magnetic agitator was maintained continuously during the addition. Also, a magnetically driven agitator was present in the surrounding ice bath to increase the rate of heat transfer from the sample bottle. To facilitate rapid settling and thus prevent interference of suspended solid particles with the conductance measurements, crystal sizes of potassium nitrate were large enough to be retained on a 35-mesh screen and small enough to pass a 10-mesh screen (Tyler Standard). The cell was moved in and out of the bottle twice in order to ensure local equilibrium within the liquid trapped inside the cell. The establishment of equilibrium by this method required about 10 minutes as indicated by the constancy of conductance with time.

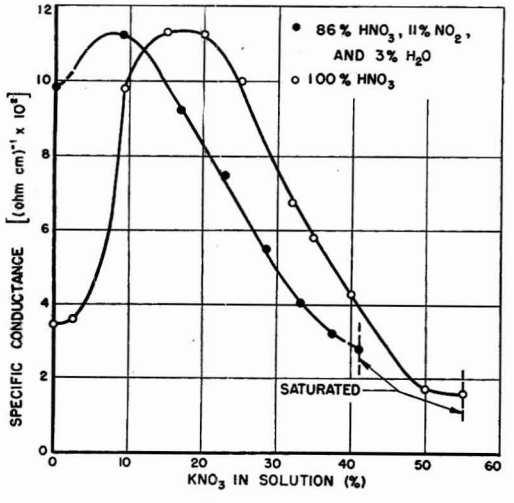

Figure 5. Effect of potassium nitrate on specific conductance of nitric acid solutions at 0° C.

In the present studies it was found that a stable salt of composition corresponding to the formula $KNO_3 \cdot 2HNO_3$ may precipitate from concentrated nitric acid solutions which are greatly supersaturated with respect to this salt. The existence of this solid phase is in keeping with the known solvation of nitrate ions (Equation 4). Moreover a compound $NH_4NO_3 \cdot 2HNO_3$ is known to form in solutions of ammonium nitrate in nitric acid (*4*). A mixture containing about 50% potassium nitrate in nitric acid was supercooled, and the resulting solid phase was placed in a specially made fritted-glass filter equipped with an external jacket through which ice water flowed. The filter fitted in a flask by means of a standard-taper glass joint, and a vacuum was applied to the flask for 1.5 hours in order to remove as much entrained liquid phase from the solid as possible. Air sucked through the filter cake previously had been dried by passing through a drying tube containing calcium chloride. The dried solid phase remaining on the filter was found to contain 56.83% nitric acid by an acidimetric titration; the remainder was assumed to be potassium nitrate. On this basis the molal ratio of nitric acid to potassium nitrate in the solid is 2.11, which closely approximates the value of 2 for the compound $KNO_3 \cdot 2HNO_3$.

Although solid anhydrous potassium nitrate, which is more soluble than the compound $KNO_3 \cdot 2HNO_3$, can apparently coexist with its solution indefinitely, it is in a metastable state which can be readily reproduced. Of course, the presence of any solid phase consisting of the compound $KNO_3 \cdot 2HNO_3$ precludes the use of the conductometric method of analysis without a knowledge of the precise amount of potassium nitrate and fuming nitric acid mixed. Care thus has to be exercised to prevent, in any portion of the liquid, excessive supersaturation which increases the tendency for the formation of the solvated stable salt. Thus potassium nitrate is added to the acid sample already cooled in the bath at 0° C. Vigorous agitation in the sample is maintained

Table I. Effect of Potassium Nitrate on Specific Conductance of Nitric Acid Solutions at 0° C.

Initial Composition of Fuming Nitric Acid, %[a]		KNO_3 in Solution, %	Specific Conductance, $(Ohm\text{-}Cm.)^{-1} \times 10^2$
NO_2	H_2O		
11	3	0	9.85
		9.1	11.22
		16.7	9.18
		23.1	7.48
		28.6	5.46
		33.3	4.04
		37.5	3.16
		41.2	2.8
		(saturated)	
0		0	3.44
		5	3.56
		9.6	9.78
		15.0	11.3
		20.0	11.2
		25.0	9.98
		32.0	6.71
		35.0	5.80
		40.0	4.28
		50.0	1.71
		55.0	1.61
		(saturated)	

[a] Remainder nitric acid to make 100%.

Table II. Experimental Values of Specific Conductance of Saturated Solutions of Potassium Nitrate in Fuming Nitric Acid at 0° C.

Composition, %[a]		Conductivity, $(Ohm\text{-}Cm.)^{-1} \times 10^2$	Composition, %[a]		Conductivity, $(Ohm\text{-}Cm.)^{-1} \times 10$
NO_2	H_2O		NO_2	H_2O	
0	0.1	1.61	11	1.5	2.16
	0.8	1.68		3.0	2.54
	3.0	2.07 2.06		4.0	2.87
	6.0	2.75 2.71	12	0	2.00
	10.0	3.84		0.1	1.85
	10.5	4.07		1.5	2.24
3	0.1	1.64		3.0	2.55
	3.0	2.07 2.19		4.0	2.90
	6.0	2.98		5.3	3.23
	9.3	4.00		6.0	3.39 3.39
4	9.2	4.00		8.4	4.28
6	0.1	1.68		10.0	4.56 4.56
	3.0	2.30 2.36 2.36	13	5.0	3.17
	5.6	3.00		6.4	3.65
	6.0	3.08	14	0	1.91 1.92 12.74[b]
	9.0	4.00		1.5	2.28
	10.0	4.33		2.9	2.65
7	0	1.76 1.76		3.8	2.85
	0.1	1.72	16	0	2.07 2.07 2.14
	2.8	2.30			13.46[b]
	3.0	2.44		1.4	2.36
	5.6	3.08		2.5	2.74
	6.0	3.23 3.23		3.0	2.79 2.83 2.81
	8.9	4.03			12.01[b] 12.15[b]
	0	1.75 1.75		3.8	3.11
8	0.1	1.73		5.0	3.34
	2.8	2.36		6.0	3.60 3.64 3.66
	3.0	2.43 2.49			11.85[b] 11.88
	5.5	3.08		8.1	4.33
	8.8	4.03		10.0	4.96 4.96
	10.0	4.50 4.50	18	0	2.23 13.79[b]
10	0.1	1.81		3.0	2.97 12.73[b]
	1.5	2.20		6.0	3.71 3.79 3.81
	2.7	2.46			12.40[b]
	3.0	2.53	20	0	2.32 14.44[b]
	5.4	3.12		3.0	3.13 13.04[b]
	8.6	4.07		6.0	3.95 4.06 12.56[b]

[a] Remainder nitric acid to make 100%.
[b] Solutions without potassium nitrate.

Table III. Smoothed Values of Specific Conductance of Saturated Solutions of Potassium Nitrate in Fuming Nitric Acid at 0° C.[a]

H_2O %	Specific Conductance for Nitrogen Dioxide, (Ohm-Cm.)$^{-1}$ × 10^2										
	0%	2.0%	4.0%	6.0%	8.0%	10.0%	12.0%	14.0%	16.0%	18.0%	20.0%
0	1.59	1.62	1.65	1.69	1.73	1.78	1.84	1.92	2.09	2.23	2.33
1	1.71	1.76	1.81	1.87	1.93	2.00	2.07	2.16	2.32	2.46	2.58
2	1.87	1.93	2.00	2.07	2.15	2.23	2.32	2.42	2.56	2.71	2.85
3	2.05	2.13	2.21	2.30	2.38	2.48	2.58	2.68	2.82	2.97	3.12
4	2.25	2.35	2.44	2.54	2.63	2.74	2.85	2.95	3.07	3.24	3.40
5	2.48	2.60	2.69	2.81	2.90	3.01	3.13	3.23	3.34	3.51	3.68
6	2.74	2.87	2.98	3.09	3.20	3.31	3.42	3.52	3.64	3.80	3.96
7	3.01	3.16	3.28	3.40	3.50	3.62	3.72	3.83	3.93		
8	3.30	3.46	3.59	3.71	3.82	3.93	4.03	4.14	4.25		
9	3.59	3.77	3.91	4.03	4.14	4.25	4.36	4.47	4.58		
10	3.90	4.10	4.25	4.37	4.48	4.59	4.69	4.81	4.92		

[a] Compositions given for nitrogen dioxide and water on a weight percentage basis, with remainder nitric acid to make 100%.

Table IV. Effect of Nitrogen Dioxide and Water in Fuming Nitric Acid on Amount of Potassium Nitrate in Saturated Solution at 0° C.

Initial Composition of Fuming Nitric Acid, %[a]		KNO_3 Solution, %
NO_2	H_2O	
0	0.8	48.9
0	6.0	42.9
16	0	44.6
16	6.0	36.5

[a] Remainder nitric acid to make 100%.

to prevent local high temperatures due to the heat of solution and consequently high concentrations of potassium nitrate in portions of the liquid which will be unduly supersaturated with respect to the compound $KNO_3 \cdot 2HNO_3$ when the portion is finally cooled to 0° C.

RESULTS

Conductance of Solutions of Potassium Nitrate in Fuming Nitric Acid. With an increase in potassium nitrate concentration in fuming nitric acid the conductance increases to a maximum and then decreases with further potassium nitrate up to the saturation point. Shown in Figure 5 and Table I are values of specific conductance as a function of percentage of potassium nitrate in the solution for one sample of 100% nitric acid and another sample containing 11% nitrogen dioxide and 3% water. The conductance rises to a peak and then decreases below its original value at the saturation point (Figure 5). The relatively slight effect of 1% potassium nitrate on the conductance indicates that the conductance is not as sensitive as might be expected to small amounts of dissolved metallic nitrates which may be present as products of corrosion in commercial acid. For example, up to 0.4% aluminum nitrate can be tolerated in fuming nitric acid containing 14% nitrogen dioxide without measurably affecting the method. However, with larger quantities of this salt measurable error is introduced into the method. In fuming nitric acid which is inhibited against corrosion, metallic salts are well below the limit which would affect this method; so this method is particularly suited to inhibited acid.

That the conductance reaches a maximum and then decreases with further addition of potassium nitrate (Figure 5) is probably due to the solvation of nitrate ions by nitric acid indicated by Equation 4. This solvation markedly increases the viscosity of the solution, which in turn lowers the mobilities of the ions and hence the conductance.

Conductance of Saturated Solutions of Potassium Nitrate in Fuming Nitric Acid. The conductance of saturated solutions of potassium nitrate in fuming nitric acid increases slightly with increasing percentage of nitrogen dioxide in the acid and markedly with increasing percentage of water. In Figure 6 and Tables II and III are shown values of the specific conductance of saturated solutions of potassium nitrate in fuming nitric acid for a range of compositions of 0 to 16% nitrogen dioxide and 0 to 10% water. Table II presents experimental data which have been subsequently smoothed in Figure 6 and Table III. Table II also contains some measurements of the conductance of samples without added potassium nitrate in the composition range, not included in (*14*), 14 to 20% nitrogen dioxide and 0 to 6% water. That an increase in nitrogen dioxide and particularly water in the acid results in an increase in the conductance of the solution is evident graphically in Figure 6. The behavior of the conductance of saturated solutions of potassium nitrate makes this property suitable as a method of analyzing fuming nitric acid.

The increased conductance of solutions of potassium nitrate in fuming nitric acid with increasing nitrogen dioxide and water accompanies a decrease in the solubility of potassium nitrate. In Figure 7 and Table IV are shown data for the amount of potas-

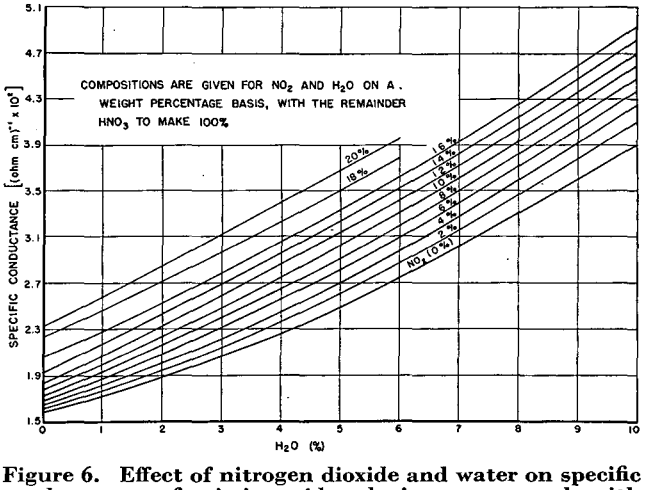

Figure 6. Effect of nitrogen dioxide and water on specific conductance of nitric acid solutions saturated with potassium nitrate at 0° C.

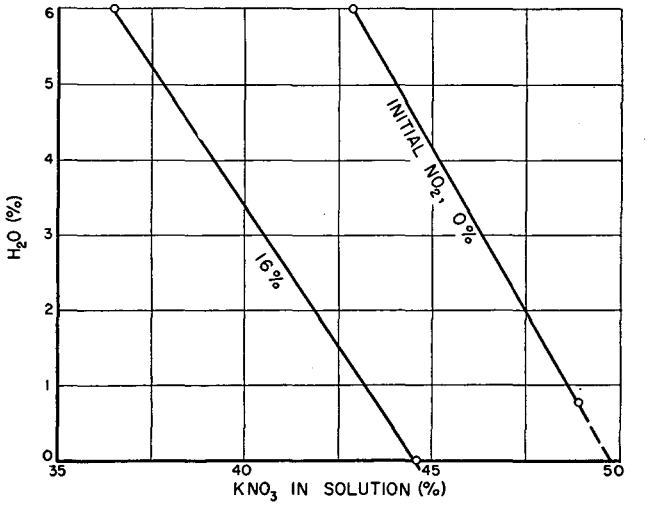

Figure 7. Effect of nitrogen dioxide and water on the amount of potassium nitrate in saturated solutions of nitric acid at 0° C.

Table V. Composition of Fuming Nitric Acid

Composition Ratio[a] $\frac{NO_2(\%)}{H_2O(\%)}$

Conductance of Fuming Nitric Acid, (Ohm-Cm.)$^{-1}$ × 10^2	For Conductance of Saturated Solution of Potassium Nitrate in Fuming Nitric Acid (Ohm-Cm.)$^{-1}$ × 10^2														
	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0
1.5				0.05	0.15	0.15	0.15	0.10							
				2.20	2.70	3.30	3.75	4.25							
2.0			0.20	0.40	0.60	0.65	0.65	0.60	0.50	0.35	0.20	0.05			
			1.60	2.10	2.65	3.15	3.60	4.15	4.60	5.05	5.45	5.90			
2.5		0.20	0.60	0.85	1.10	1.20	1.20	1.20	1.10	1.00	0.85	0.70	0.50	0.25	0.00
		0.90	1.50	2.05	1.50	3.05	3.50	4.00	4.45	4.90	5.30	5.70	6.10	6.55	6.95
3.0		0.55	1.05	1.40	1.60	1.75	1.85	1.85	1.80	1.70	1.60	1.40	1.20	1.00	0.70
		0.85	1.40	1.90	2.40	2.90	3.35	3.85	4.30	4.75	5.15	5.55	6.00	6.35	6.80
3.5	0.20	1.15	1.70	2.05	2.25	2.35	2.40	2.40	2.35	2.30	2.20	2.05	1.85	1.65	1.40
	0.10	0.75	1.30	1.80	2.30	2.80	3.75	3.70	4.15	4.60	5.00	5.40	5.85	6.20	6.60
4.0	0.50	1.55	2.25	2.70	3.00	3.10	3.15	3.10	3.00	2.90	2.80	2.65	2.40	2.25	2.00
	0	0.70	1.20	1.65	2.15	2.65	3.10	3.60	4.05	4.45	4.85	5.25	5.70	6.10	6.50
4.5		2.15	2.80	3.20	3.45	3.60	3.70	3.70	3.70	3.60	3.50	3.35	3.20	3.00	2.75
		0.60	1.10	1.60	2.10	2.55	2.95	3.45	3.90	4.30	4.70	5.10	5.55	5.90	6.30
5.0		2.60	3.25	3.65	3.95	4.10	4.25	4.35	4.40	4.35	4.30	4.25	4.10	4.00	3.80
		0.50	1.05	1.50	2.00	2.45	2.85	3.30	3.75	4.15	4.50	4.90	5.35	5.70	6.10
5.5		3.10	3.70	4.10	4.40	4.60	4.75	4.80	4.85	4.80	4.80	4.75	4.65	4.60	4.45
		0.45	1.00	1.40	1.90	2.35	2.80	3.20	3.65	4.10	4.40	4.80	5.25	5.60	5.95
6.0		3.65	4.25	4.65	4.90	5.10	5.20	5.30	5.30	5.30	5.30	5.25	5.20	5.15	5.10
		0.35	0.90	1.35	1.80	2.25	2.70	3.10	3.55	3.95	4.30	4.70	5.10	5.45	5.80
6.5		4.20	4.80	5.15	5.35	5.55	5.70	5.80	5.90	5.90	5.90	5.85	5.80	5.70	5.65
		0.30	0.80	1.30	1.75	2.15	2.60	3.00	3.45	3.85	4.20	4.60	5.00	5.35	5.70
7.0		4.80	5.30	5.70	5.95	6.10	6.25	6.35	6.45	6.50	6.50	6.50	6.50	6.45	6.40
		0.20	0.75	1.20	1.65	2.10	2.50	2.90	3.30	3.75	4.10	4.45	4.85	5.20	5.60
7.5		5.35	5.85	6.20	6.55	6.75	6.90	7.10	7.20	7.25	7.30	7.30	7.80	7.25	7.20
		0.15	0.70	1.10	1.55	1.95	2.40	2.80	3.20	3.60	3.90	4.30	4.70	5.10	5.40
8.0		6.05	6.50	6.80	7.15	7.35	7.60	7.75	7.85	8.00	8.05	8.10	8.10	8.05	8.00
		0.05	0.60	1.00	1.45	1.85	2.25	2.65	3.05	3.45	3.80	4.15	4.55	4.95	5.30
8.5			7.15	7.45	7.75	8.05	8.25	8.40	8.60	8.70	8.80	8.80	8.85	8.85	8.85
			0.50	0.90	1.35	1.75	2.15	2.55	2.90	3.30	3.65	4.00	4.40	4.80	5.15
9.0			7.80	8.15	8.45	8.75	8.95	9.20	9.35	9.50	9.60	9.70	9.70	9.70	9.70
			0.40	0.85	1.20	1.70	2.05	2.40	2.80	3.20	3.55	3.85	4.25	4.65	5.00
9.5			8.40	8.80	9.15	9.45	9.70	9.90	10.15	10.30	10.40	10.50	10.60	10.60	10.65
			0.30	0.70	1.15	1.55	1.90	2.30	2.65	3.05	3.40	3.70	4.10	4.50	4.85
10.0			9.20	9.60	9.90	10.20	10.45	10.70	10.90	11.10	11.25	11.35	11.45	11.55	11.60
			0.20	0.60	1.00	1.45	1.75	2.15	2.55	2.90	3.25	3.60	3.95	4.30	4.65
10.5			9.95	10.30	10.65	10.95	11.25	11.50	11.75	11.95	12.15	12.30	12.45	12.55	12.60
			0.10	0.50	0.90	1.30	1.65	2.00	2.40	2.75	3.05	3.40	3.80	4.15	4.50
11.0			10.75	11.10	11.35	11.65	11.90	12.20	12.45	12.70	12.90	13.15	13.35	13.55	13.70
			0.00	0.40	0.80	1.20	1.55	1.90	2.25	2.60	2.95	3.30	3.65	3.95	4.30
11.5				11.85	12.20	12.55	12.85	13.15	13.45	13.70	13.95	14.20	14.40	14.65	14.70
				0.30	0.65	1.05	1.40	1.70	2.10	2.40	2.75	3.10	3.45	3.75	4.05
12.0				12.80	12.30	13.55	13.90	14.25	14.55	14.75	15.05	15.30	15.60	15.85	16.05
				0.15	0.65	0.85	1.20	1.45	1.80	2.10	2.35	2.70	3.05	3.40	3.70
12.5				13.65	14.00	14.40	14.80	15.20	15.55	15.85	16.20	16.50	16.80	17.05	17.30
				0	0.30	0.60	0.90	1.20	1.50	1.80	2.10	2.40	2.75	3.05	3.35
13.0					14.85	15.20	15.65	16.10	16.60	16.95	17.45	17.90	18.35	18.80	19.20
					0.05	0.30	0.60	0.90	1.30	1.45	1.80	2.05	2.30	2.55	2.75
13.5						16.00	16.65	17.35	17.90	18.40	18.95	19.55	20.00		
						0	0.25	0.65	0.85	1.10	1.35	1.60	1.80		
14.0							18.85	19.40	19.80						
							0.15	0.45	0.70						

[a] Remainder nitric acid to make 100%.

from a Pair of Conductance Measurements at 0° C.

Composition Ratio[a] $\frac{NO_2(\%)}{H_2O(\%)}$															Conductance of Fuming Nitric Acid, (Ohm-Cm.)$^{-1}$ × 10^2
For Conductance of Saturated Solution of Potassium Nitrate in Fuming Nitric Acid (Ohm-Cm.)$^{-1}$ × 10^2															
3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	1.5
															2.0
															2.5
0.40 / 7.20	0.10 / 7.65														3.0
1.15 / 7.05	0.90 / 7.45	0.60 / 7.80	0.25 / 8.25												3.5
1.75 / 6.90	1.55 / 7.30	1.25 / 7.65	1.00 / 8.05	0.70 / 8.50	0.40 / 8.90	0.10 / 9.30									4.0
2.55 / 6.70	2.30 / 7.10	2.05 / 7.45	1.75 / 7.85	1.45 / 8.30	1.10 / 8.70	0.80 / 9.10	0.40 / 9.60	0.05 / 9.95							4.5
3.60 / 6.50	3.35 / 6.85	3.10 / 7.20	2.80 / 7.60	2.50 / 8.00	2.15 / 8.40	1.80 / 8.80	1.35 / 9.30	1.00 / 9.70							5.0
4.30 / 6.30	4.10 / 6.70	3.90 / 7.05	3.65 / 7.40	3.30 / 7.85	3.05 / 8.20	2.70 / 8.60	2.25 / 9.05	1.90 / 9.45	1.40 / 9.90						5.5
4.95 / 6.20	4.80 / 6.55	4.60 / 6.90	4.45 / 7.25	4.20 / 7.65	3.95 / 8.00	3.70 / 8.35	3.35 / 8.80	3.10 / 9.15	2.75 / 9.55	2.45 / 9.90					6.0
5.50 / 6.10	5.40 / 6.40	5.20 / 6.80	5.05 / 7.10	4.80 / 7.50	4.65 / 7.85	4.40 / 8.20	4.15 / 8.60	3.85 / 9.00	3.60 / 9.30	3.30 / 9.70					6.5
6.25 / 5.95	6.20 / 6.30	6.05 / 6.60	5.85 / 6.95	5.70 / 7.35	5.50 / 7.70	5.25 / 8.05	5.05 / 8.40	4.75 / 8.80	4.50 / 9.15	4.25 / 9.50	3.95 / 9.85				7.0
7.10 / 5.80	7.00 / 6.10	6.85 / 6.50	6.75 / 6.80	6.55 / 7.20	6.35 / 7.50	6.10 / 7.90	5.85 / 8.25	5.65 / 8.60	5.40 / 8.95	4.10 / 9.30	4.80 / 9.70				7.5
7.95 / 5.65	7.85 / 6.00	7.75 / 6.30	7.60 / 6.65	7.45 / 7.05	7.30 / 7.35	7.10 / 7.70	6.85 / 8.10	6.60 / 8.40	6.40 / 8.80	6.15 / 9.10	5.90 / 9.50	5.60 / 9.90			8.0
8.75 / 5.50	8.70 / 5.80	8.60 / 6.20	8.45 / 6.50	8.30 / 6.90	8.15 / 7.20	7.95 / 7.55	7.75 / 7.95	7.55 / 8.30	7.30 / 8.60	7.05 / 8.95	6.80 / 9.30	6.50 / 9.70			8.5
9.70 / 5.30	9.60 / 5.70	9.50 / 6.05	9.40 / 6.35	9.30 / 6.70	9.10 / 7.10	8.95 / 7.40	8.75 / 7.80	8.55 / 8.10	8.30 / 8.45	8.10 / 8.80	7.85 / 9.15	7.60 / 9.50	7.35 / 9.85		9.0
10.65 / 5.15	10.60 / 5.55	10.55 / 5.85	10.50 / 6.20	10.35 / 6.55	10.20 / 6.90	10.10 / 7.20	9.90 / 7.60	9.70 / 7.90	9.50 / 8.25	9.30 / 8.60	9.10 / 8.90	9.80 / 9.30	8.60 / 9.65		9.5
11.60 / 5.00	11.60 / 5.40	11.60 / 5.70	11.55 / 6.05	11.45 / 6.40	11.35 / 6.75	11.25 / 7.05	11.10 / 7.40	10.90 / 7.75	10.70 / 8.05	10.50 / 8.40	10.25 / 8.70	10.00 / 9.10	9.75 / 9.45	9.45 / 9.80	10.0
12.65 / 4.80	12.70 / 5.20	12.75 / 5.50	12.75 / 5.80												10.5
13.80 / 4.60	13.90 / 5.00	14.00 / 5.30	14.00 / 5.60	14.00 / 6.00											11.0
14.85 / 4.40	14.95 / 4.75	15.05 / 5.05	15.15 / 5.40	15.20 / 5.45											11.5
16.25 / 4.05	16.35 / 4.35	16.45 / 4.70	16.55 / 5.00	16.60 / 5.40	16.65 / 5.70	16.70 / 6.05									12.0
17.50 / 3.65	17.75 / 3.95	17.95 / 4.25	18.15 / 4.55	18.35 / 4.80	18.60 / 5.15	18.75 / 5.45	18.95 / 5.70	19.15 / 6.00							12.5
19.65 / 3.00															13.0
															13.5
															14.0

sium nitrate in a saturated solution of fuming nitric acid for several initial compositions. With increasing percentage of nitrogen dioxide and water the solubility of potassium nitrate decreases, and this decrease in solubility is most markedly affected by water. Figure 5 and Table I show that in concentrated solutions of potassium nitrate an increase in the amount of potassium nitrate in solution decreases the conductance. Thus the rise in conductance with increase in nitrogen dioxide and water can be attributed to the decrease in solubility of potassium nitrate with an increase in nitrogen dioxide and water.

Analysis of Fuming Nitric Acid by Pair of Conductance Measurements. The set of intersecting curves of constant conductance in Figure 1 or the corresponding data in Table V may be used to determine the composition of an unknown sample of fuming nitric acid. The conductance of the sample without potassium nitrate is measured at 0° C. in equipment shown in Figure 4. The conductance of a duplicate sample saturated with potassium nitrate is measured at 0° C. as described. After the conductance of these duplicate samples has been made, the composition of the sample may be determined from the data of Figure 1 or Table V in the following manner: If, for example, the conductance at 0° C. on an unknown sample is 11.0×10^{-2} ohm per cm. and on the same sample saturated with potassium nitrate is 2.2×10^{-2} ohms per cm., the composition of the sample is given in Figure 1 at the intersection of the appropriate pair of curves of constant conductance. Alternately the value of composition is given at the intersection of the two appropriate columns of constant conductance in Table V. Linear extrapolation may be used with these data. The composition of the original sample is seen from these data to be about 11.9% nitrogen dioxide and 1.6% water, the remainder from 100 being 86.5% nitric acid. By this procedure an analysis of a sample of fuming nitric acid of unknown composition can be determined within 15 minutes.

CONCLUSIONS

With the conductometric method employing saturated solutions of potassium nitrate, impurities in commercial acid do not introduce as much error in the determination of water (in low concentrations) as does the conventional chemical analysis in which water is determined by difference. In principle the use of equilibrium between solid and liquid phases might be of general applicability for the conductometric analysis of electrolytes where gravimetric or volumetric measurements are to be avoided. Although the present measurements were made for convenience at the ice point, the method could be extended to be used at other temperatures. With the techniques outlined, this conductometric method of analysis gives the composition of fuming nitric acid to ±0.3% for both nitrogen dioxide and water over the range of composition studied. If higher accuracy is required, refinements in the conductance bridge and in the chemical techniques could be made. However, the convenience and rapidity of the method make it desirable for the analysis of the nitric acid–nitrogen dioxide–water system whenever rapidity at the expense of high accuracy is desired.

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Preparation and Standardization of Perchloratoceric Acid Solutions in Perchloric Acid

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Laboratory directions for the preparation of cerium(IV) solutions in perchloric acid as supporting electrolyte are given, including directions for standardization. The process is direct and efficient, and requires only readily available laboratory equipment and chemicals. It is practical in routine application and provides an important analytical reagent of extraordinarily high oxidation potential. As the reagent is stable for only a limited storage time, its preparation in small quantities is a noteworthy advantage.

SOLUTIONS of perchloratoceric acid in various strengths of perchloric acid (*5*) have found many important uses in a wide field of analytical applications. The present work describes a convenient method for their preparation and a procedure for their precise standardization. The possible objection of the presence of nitrates has been eliminated.

HALF-CELL REACTION POTENTIALS OF PERCHLORATOCERIC ACID SOLUTIONS IN PERCHLORIC ACID

The oxidation potentials (*3*, *5*) of the cerium(IV)—cerium (III) couple in the half cell

$$Ce(IV) + e^- = Ce(III)\ \text{(acidified with perchloric acid)}$$

are the following: $1F = 1.71$, $2F = 1.73$, $4F = 1.78$, $6F = 1.83$, and $8F = 1.87$ volts. At these unusually high voltage values the following reactions illustrate the mechanism of oxidation involved and explain the very slow, but detectable, instability of perchloratoceric acid with water as the second reacting component.

$$4H{:}\ddot{O}{:}H + 4H{:}\ddot{O}{:}H + 8Ce(IV) = 4(\ddot{O}{:}H)^+ + 4(:\ddot{O}{:}H)^- + 8H^+ + 8Ce(III)$$

$$4(:\ddot{O}{:}H)^- + 4H^+ = 4H_2O$$

$$4(\ddot{O}{:}H)^+ = 2H_2O + O_2$$

These reactions normally involve a modest rate, but they may be catalyzed by the presence of platinum. At room temperature, if 500 mg. of platinum sponge is stirred in contact with 250 ml. of a $0.2N$ solution of perchloratoceric acid in $2F$ perchloric acid (oxidation potential 1.73 volts) for 60 minutes, over 50% of the cerium(IV) is reduced to cerium(III). Even with the same amount of platinum in the form of sheet or wire, with much reduced surface area, the decomposition is appreciable. Graphite in the form of rods (such as the electrodes employed for spectrographic emission operations) can duplicate platinum sponge in the accelerated reduction of cerium(IV) in perchloric acid solution.

The values of oxidation potential (*5*) provided by perchloric acid solutions of cerium (IV) are high enough to be compared with such high values as those of sodium persulfate^{--}, ozone, and the bismuthate ion, which are not employed in quantitative operations involving standard solutions of these oxidants. Solutions of cerium(IV) in perchloric acid are as stable as (*5*), or more stable than corresponding solutions of the permanganate ion employed as oxidant, at a much lower oxidation potential rating. Standard solutions of cerium(IV) in perchloric acid retain their original oxidation values better when kept at 40° F. in the absence of light—for example, by refrigerator storage—than when storage is at ordinary temperatures and in diffuse daylight (*11*). In the latter case restandardization at 72-hour intervals is requisite for the most precise applications.

PERCHLORATOCERIC ACID APPLICATIONS IN ANALYSIS

An important application of perchloratoceric acid is in qualitative organic analysis in the identification of hydroxy groups and has been described by Shriner and Fuson (*4*) based upon the original description of the process by Duke and Smith (*2*). The determination of glycerol, following its oxidation to formic acid by perchloratoceric acid, as well as the perchloratoceric acid determination of many other organic compounds has been described by Smith and Duke (*6*, *7*). The micro determination of calcium in blood has been described by Salamon, Gabrio, and Smith (*3*), and of oxalic acid, iron, and arsenic by Smith and Fritz (*10*). In all these applications the reactions were found to be stoichiometric. Nitro-1,10-phenanthroline–ferrous sulfate is employed as indicator because of its high oxidation potential (*5*).

AMMONIUM NITRATOCERATE AS STARTING MATERIAL IN PREPARATION OF PERCHLORATOCERIC ACID

Ammonium nitratocerate is commercially available both as reagent grade (98.0 to 99.5% pure) and as a primary standard for use in oxidimetry (purity 99.98 to 100.00%) (*8*). It is soluble in water if the solution is maintained saturated (pH approximately 1). Such solutions of ammonium nitratocerate are instantly reduced by hydrogen peroxide to ammonium nitrate, cerium (III) nitrate, and nitric acid. With an excess of concentrated hydrochloric acid these reaction products produce, on boiling, a solution of cerium (III) chloride in hydrochloric acid medium. By boiling with excess perchloric acid, the conversion to cerium(III) perchlorate is readily accomplished. The last stage in the preparation of an approximately $1.0F$ solution of cerium(IV), in an approximately $1.0F$ solution of perchloric acid, involves electrooxidation. For this purpose there are applied a platinum anode and a platinum cathode. No partition cell is required (*9*). The solution thus prepared may then be readily (by appropriate dilution with perchloric acid and water) altered to give any desired strength of oxidizing reactant at any selected acid concentration. One pound of the starting material, hexanitratoammonium cerate, is equivalent to somewhat more than 115 grams of cerium. Over 16,400 ml. of an approximately $0.05N$ solution of cerium(IV) are thus provided for.

Apparatus. The manipulations outlined above are best carried out using the digestion apparatus, shown in Figure 1, which eliminates the required use of a forced-draft fume hood and can be employed on any laboratory work bench beside a sink drain, supplied with pressure tap water and a good metal aspirator pump. The use of a 500-ml. 96% silica glass flask is highly recommended for carrying out the digestion reactions.

Figure 1. Digestion apparatus with fume eradicator

The apparatus assembly shown in Figure 1 consists of a 1000-ml. reduced pressure filtering flask to which is attached a modified 500- or 800-ml. Kjeldahl digestion flask serving as a fume eradicator. The aspirator flask may be filled with 800 ml. of strong sodium hydroxide for the absorption of evolved acid fumes, and the aspirator pump should provide a liberal exhaust of air at the neck of the reaction flask to prevent escape of acid fumes from the digesting solution in the reaction flask. The electric hot plate should be provided with a variable heat control regulating device for convenience in controlling boiling rates.

In the assembled apparatus for the electro-oxidation of cerium (III) to cerium(IV), an electromagnetic stirring device serves as a support for a 400-ml. reaction beaker containing the cerium(III) solution. A platinum gauze anode, approximately 50 by 75 mm. is suspended in the center of the beaker and is elevated 5 mm. above the bottom of the beaker to provide room for the stirring rotor. A 100-mm. length of 0.5-mm. platinum wire is suspended along the inner side of the reaction beaker to serve as cathode. A 6-volt dry cell serves to supply the electric energy. This assembled apparatus requires no attention. Three 1.5-volt dry batteries are of sufficient capacity and potential to oxidize the cerium from 200 grams of hexanitratoammonium cerate.

Procedure. Place 220 grams of hexanitratoammonium cerate in a 500-ml. Erlenmeyer flask and dissolve in 120 ml. of water at 60° C. Add 25 ml. of 100-volume (30%) hydrogen peroxide in small portions to reduce cerium(IV) to cerium(III) and produce a colorless solution. Place the flask and contents on the hot plate and heat to boiling (Figure 1). Add 500 ml. of concentrated hydrochloric acid (specific gravity 1.19) in 25-ml. portions with intermediate boiling. Maintain the original reaction volume by

the stepwise addition of hydrochloric acid until all 500 ml. has been added. At the end allow the solution to concentrate to approximately 200 ml.

Add a few pieces of Carborundum boiling chips and 240 ml. of 70% perchloric acid. (If the solution turns red and produces chlorine fumes, all nitric acid has not been removed.) Boil briskly until copious fumes of perchloric acid are evolved, all hydrochloric acid has been displaced, and the cerium(III) chloride has been converted completely to cerium(III) perchlorate. The solution when hot will have a light yellow color but when cool should be colorless.

Transfer the cold solution, diluted to 500 ml., to the oxidation beaker. Connect the electrodes to the proper battery poles and start the stirrer rotating. The apparatus requires no further attention, and the oxidation is complete in 20 to 24 hours. The final solution, when finally diluted to 600 ml., will be approximately 1*F* cerium(IV) in 1*F* perchloric acid and will be orange in color. Store in a glass-stoppered bottle in the icebox until ready for dilution with 1*F* perchloric acid to the strength of solution desired.

SODIUM OXALATE AS PRIMARY STANDARD FOR STANDARDIZATION OF PERCHLORATOCERIC ACID

National Bureau of Standards sodium oxalate (sample 40c or of equivalent purity) is employed after drying at 110° C. If an approximately 0.1*N* solution of cerium(IV) is to be evaluated, weigh samples of 0.2 to 0.3 gram, correct to 0.1 mg., and transfer them to 400-ml. beakers. Dissolve the sodium oxalate in 150 ml. of 1*F* perchloric acid. Titrate the oxalate employing the unknown cerium(IV) solution until most of the colored cerium(IV) has been reduced. Add a drop of 0.025*M* nitroferroin (5-nitro-1,10-phenanthroline ferrous sulfate) and complete the reaction to the disappearance of the pink color. The reaction is carried out at ordinary temperatures. Electromagnetic stirring is recommended. When first added, the indicator will precipitate as the iron(II) perchlorate complex, but this precipitate dissolves after a few seconds' stirring. There is little warning of the approach of the equivalence point and for this reason a titration thief (*1*) is employed to advantage.

Solutions of perchloratoceric acid [1*F* in cerium(IV) and 6*F* in perchloric acid] are commercially available.

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Estimation of Silicon in the Presence of Fluorine

Application to Phosphate Rock and Wet-Process Phosphoric Acid

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The fluorine in a sample of phosphate rock or phosphoric acid is distilled from a perchloric acid solution to which has been added a known quantity of silica as sodium silicate solution. The silicon in the distillate is free from interfering elements and is determined volumetrically. The silica remaining in the flask is substantially free from fluorine and is determined gravimetrically. For accurate work on phosphate rock an intermediate alkaline fusion and second distillation are added. The silicate solution is added to prevent attack on the glass by fluorine. Suitable quantities are 2.5 to 5 grams of phosphate rock, or phosphoric acid containing about 100 mg. of fluorine, and 200 to 400 mg. of added silica. An accuracy of 1 to 2 mg. of silica was obtained on blank determinations. On standard rock using the routine procedure results within 5 mg. of the stated value were obtained, and using the accurate procedure results were within 2 mg. A routine determination can be finished within 6 hours. An alternative procedure is based on separation of silica from phosphate by precipitation as potassium silicofluoride, followed by gravimetric estimation as silicomolybdate.

THE classical method for the determination of silica fails in the presence of fluorine because of losses due to silicon tetrafluoride. When boric acid is added (*2*) better results are obtained, but they are still low and nonreproducible (*8*). The method generally described in the literature for the estimation of silicon in the presence of fluorine is the method of Berzelius, modified by various workers (*3–5*) which is based on the precipitation of silica with zinc oxide in alkaline and neutral solution; this procedure is very complicated. The simplification of Shell and Craig fails in the presence of phosphate and calcium ions (*11*). Other methods based on the formation of the silicomolybdate complex (*1*) are not suitable for materials containing a large excess of phosphate over silica. It was therefore decided to look for a new method rather than to modify further the above methods.

Two methods were investigated, one based on the precipitation of the silica as silicofluoride, the second on distillation. During the course of the work the distillation method appeared more promising, and the work on the silicofluoride method was not continued; it is reported here only in outline.

POTASSIUM SILICOFLUORIDE METHOD

Travers (*12*) determined silica by precipitation as potassium silicofluoride in the presence of hydrofluoric acid and a large excess of potassium chloride. After filtering and washing, he titrated the precipitate with alkali according to the reaction:

$$K_2SiF_6 + 4NaOH \rightarrow 2KF + 4NaF + Si(OH)_4$$

In the present work it was found that the solubility of potassium silicofluoride in water at 25° C. fell from 130 to 8 mg. per 100 ml. by the addition of 5% potassium chloride, and further additions reduced the solubility only slightly. However, the further addition of 0.5% potassium fluoride reduced the solubility to 2 mg. per 100 ml., and there is evidence that at pH 3 it is considerably lower. These results are in agreement with the assumption

that the silicofluoride ion hydrolyzes partially according to the equation

$$SiF_6^{--} + 4H_2O \leftrightharpoons Si(OH)_4 + 4HF + 2F^-$$

and that in order to reduce the solubility to a minimum, the hydrolysis must be suppressed by the presence of fluoride ions and hydrofluoric acid.

Jacobson (*6*) reports that the titration of silicofluoride requires more than the theoretical amount of alkali, presumably due to ionization of silicic acid. In the present work a titration factor was used, obtained by titrating analytical reagent grade sodium silicofluoride.

Known solutions of sodium silicate, with or without addition of large amounts of phosphoric acid, were analyzed by the following procedure and gave results with a maximum error of 0.8%.

EXPERIMENTAL PROCEDURE

Place a sample, containing 50 to 250 mg. of silica in solution, in a plastic beaker and dilute to 200 ml. Add 3 grams of potassium fluoride, 10 grams of potassium chloride, and hydrochloric acid until distinctly acid to methyl orange. Stir and let stand for 1 hour. Filter through a retentive quantitative paper in a plastic filter funnel, and wash with a solution prepared by dissolving 150 grams of potassium chloride and 15 grams of potassium fluoride in 3 liters of water. Add 0.5 gram of potassium silicofluoride, stir for 30 minutes, and filter. Continue washing until the alkaline titer of a heated sample of the filtrate is the same as that of an equal volume of the wash solution. Transfer precipitate and paper to an Erlenmeyer flask. Rinse out the plastic beaker with 300 ml. of hot water. Titrate at 80° C. with sodium hydroxide solution, using phenolphthalein as indicator, to the first permanent appearance of color. Correct for the silica in the reagents by a blank determination.

Phosphate rock and technical grade phosphoric acid were tested by the above procedure. [Additional potassium fluoride, sufficient to combine with all fluorine complexing elements, was required by some samples. Phosphate rock was brought into solution by the method of Brabson and coworkers (*1*).] The titration failed, however, because of interference from aluminum and iron(III) which precipitated together with the silicon. Experiments were made to determine the silicon in the mixed precipitate, which should be free from phosphate, gravimetrically as the silicomolybdate (*1*). Preliminary results gave errors of up to 5%, and the work was discontinued in favor of the simpler distillation method.

PROPOSED DISTILLATION METHOD

According to Willard and Winter (*13*), fluorine can be distilled quantitatively in the presence of silica from an acid solution at 135° C. Thus, at the end of the distillation the residue will be free from fluorine, and the silica in it can be determined gravimetrically. The distillate will not contain any interfering elements, and its silicon can be determined volumetrically. Fluorine attacks freshly precipitated silica in preference to the glass of the apparatus.

The sample of phosphate rock or phosphoric acid, together with perchloric acid and a known addition of silica as sodium silicate, is placed in the distilling flask and steam-distilled.

Silicon Remaining in Distilling Flask. The silica remaining in the distilling flask is found entirely as precipitate. This was confirmed several times by filtering, evaporating the filtrate until fumes of perchloric acid appeared, and refluxing for 20 minutes. No silica precipitated. However, when silica was determined gravimetrically in sodium silicate solution in the absence of fluorine, some silica remained in solution after the first precipitation, in agreement with data in the literature. The explanation may be that the distilling fluorine removes any silicon remaining in solution.

The silica is filtered, washed, ignited, and weighed. The weight of the impurities remaining after removal of the silica with a mixture of hydrofluoric and nitric acids is deducted from the weight of silica.

The residue from the distillation of phosphate rock may include undecomposed silicates and fluorides, either of which may introduce a small error into the results. For accurate work this residue should be fused with alkali and redistilled. This step is not required in the analysis of phosphoric acid.

Silicon in Distillate. In the condensate the following reactions take place:

$$3SiF_4 + 4H_2O \rightarrow 2H_2SiF_6 + Si(OH)_4 \quad (1)$$
$$6HF + Si(OH)_4 \rightarrow H_2SiF_6 + 4H_2O \quad (2)$$

Thus in addition to fluosilicic acid and any volatile acid from the sample, the distillate will also contain either free silicic acid or free hydrofluoric acid.

The silicon is determined according to the following reactions:

$$Si(OH)_4 + 6F^- + 4H^+ \rightarrow SiF_6^{--} + 4H_2O \quad (3)$$
(in cold acid solution)
$$SiF_6^{--} + 4OH^- \rightarrow 6F^- + Si(OH)_4 \quad (4)$$
(in hot alkaline solution)

The conditions under which Reaction 3 occurs have been examined by Sawaya (*10*).

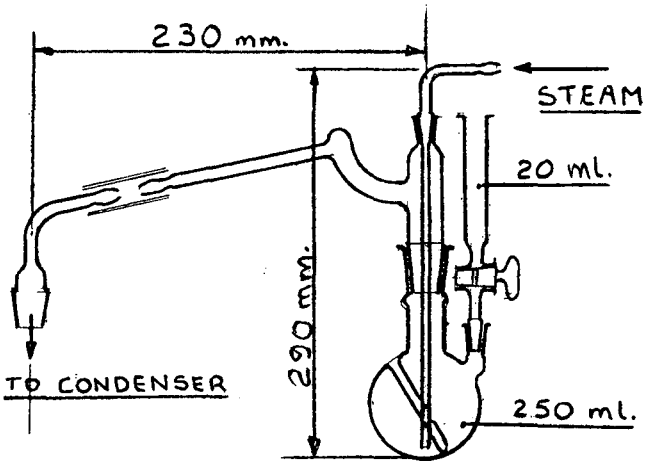

Figure 1. Distillation apparatus for silica determination

In an aliquot of the distillate any free silicic acid is converted to potassium silicofluoride according to Reaction 3. The free acid is then neutralized and the silicon, which is now present only as silicofluoride, is determined according to Reaction 4. In order to prevent reversal of Reaction 3 during the neutralization, the solution is cooled and excess potassium chloride added which precipitates nearly all the silicofluoride.

Since the reagents may contain a little silica and since slightly more than the theoretical quantity of sodium hydroxide is required in the silicofluoride titration, accurate work requires the determination both of a blank and of an experimental titration factor. Without these precautions the titration gave a positive error of about 1 mg. on an aliquot containing 20 mg. of silica. The basis of this titration is also discussed with the potassium silicofluoride method.

Estimation of Fluorine. The fluorine in the distillate was determined by the thorium nitrate method as modified by Pietzka and Ehrlich (*9*).

Choice and Quantity of Silicon to Be Added. Table I gives the results of experiments in which fluorine was distilled from analytical reagent grade sodium fluoride and perchloric acid, either without any additions or with the addition of finely powdered quartz or sodium silicate solution.

The quantity of silicon in the distillate per gram of fluorine distilling does not vary much. However, with no addition or with

addition of quartz, the silicon is derived almost entirely from the glass apparatus; in Experiment 3 the quartz added was practically unattacked. When 160 mg. or more of silica as sodium silicate solution were added, the recovery was practically theoretical, showing that in this case little or no attack on the glassware occurred.

APPARATUS AND REAGENTS

Distilling flask with steam generator and condenser (Figure 1).

Reagents. All analytical reagent grade.

SODIUM SILICATE SOLUTION A. Dissolve 50 grams of sodium silicate in 800 ml. of hot water with the addition of a few pellets of sodium hydroxide. Cool, let stand 1 day, filter, dilute to 1 liter, and store in a plastic bottle. Determine the silica content gravimetrically.

SODIUM SILICATE SOLUTION B. Dilute 25 ml. of solution A to 250 ml. Store in plastic bottle.

HYDROCHLORIC ACID SOLUTION, 0.2*N*.

SODIUM HYDROXIDE SOLUTION, 0.2*N*, free of carbon dioxide and silica. Store in a plastic bottle. Empty buret each day after use to avoid contamination with silica.

POTASSIUM FLUORIDE SOLUTION. Dissolve 30 grams of potassium fluoride in 400 ml. of water. Dilute to 500 ml. Store in plastic bottle.

PROCEDURE A (ROUTINE ANALYSIS)

In the distilling flask place 2.5 to 5 grams of phosphate rock plus 15 ml. of water, or a sample of phosphoric acid containing about 100 mg. of fluorine. Add 10 ml. of sodium silicate solution A, assemble apparatus, and add 25 ml. of 70% perchloric acid through the dropping funnel. (When analyzing phosphoric acid low in calcium, sulfuric acid may replace perchloric acid.) Start a slow stream of steam, and heat the flask to 110° to 120° C. Collect the distillate in a 500-ml. plastic beaker. After 50 ml. has been collected, raise the temperature to 135°C. and continue until the volume of distillate is about 450 ml. This should take about 90 minutes. Transfer to a 500-ml. volumetric flask, rinse the beaker, and dilute to the mark. Mix and immediately transfer to a plastic bottle. (The usual precautions should be taken when using perchloric acid.)

Determination of Silica in Distilling Flask. After the distillation is completed, disassemble the apparatus and wash the contents into a beaker. Filter through a coarse quantitative paper, wash until filtrate shows no acidity, ignite in a platinum crucible, and weigh. Add hydrofluoric acid and a few drops of nitric acid, evaporate to dryness, ignite, and weigh. The difference in weight is taken as the silica in the flask.

Determination of Silicon in Distillate. Measure into a plastic beaker a 200-ml. aliquot of the distillate. Add 3 drops of phenolphthalein (0.5%), 20 ml. of 0.2*N* hydrochloric acid, 10 grams of potassium chloride, and 10 ml. of potassium fluoride solution. Stir with a plastic rod until the potassium chloride dissolves and cool the solution to 0° C. Neutralize rapidly with 0.2*N* sodium hydroxide solution to a pink color persisting for 10 seconds. Transfer to an Erlenmeyer flask, and rinse out the beaker with hot water. Heat to 80° C. and titrate to a faint permanent pink color.

$$\text{Mg. of } SiO_2 \text{ in aliquot} = 3.00 \times \text{ml. of } 0.2N \text{ NaOH}$$

PROCEDURE B (ACCURATE ANALYSIS)

Distill sample from perchloric acid as in Procedure A.

Determination of Silica in Distilling Flask. When analyzing phosphoric acid or a soluble silicate, proceed as in Procedure A. For phosphate rock, collect and filter the silica and unattacked silicates as in Procedure A. Dry the filter at 105° C., remove the precipitate from the paper, and burn the paper in a platinum dish. Transfer the precipitate to the platinum dish, add 2 grams of sodium carbonate, mix well, cover with a thin layer of sodium carbonate, and heat till a clear melt is obtained. Cool and dissolve the melt in hot water. Transfer to the distilling flask, add immediately 25 ml. of perchloric or sulfuric acid, and repeat the distillation as in Procedure A, but without any further addition of sodium silicate. Determine silica in distilling flask as in Procedure A.

Determination of Silicon in Distillates. Make a blank titration according to Procedure A, using 200 ml. of water in place of the distillate in order to determine the silica in the reagents (*a* milliliters of sodium hydroxide). Take 10 ml. of sodium silicate solution B, dilute to 200 ml., and titrate as in Procedure A (*b* milliliters of sodium hydroxide). Titrate a 200-ml. aliquot of the distillate to be analyzed (*c* milliliters of sodium hydroxide). If the standard silicate solution B contains *s* milligrams of silica per 10 ml.,

$$\text{Mg. of silica in aliquot} = s \times \frac{c - a}{b - a}$$

RESULTS AND DISCUSSION

Experiments 4 to 10 in Table I show that a certain minimum of silica has to be added to prevent attack on the glass apparatus.

Table I. Determination of Silicon

(Fluorine distilled from sodium fluoride with or without added quartz or sodium silicate[a])

	Taken			Found				
No.	Form of silicon	SiO_2, Gram	F, gram	F, gram	Flask SiO_2, gram	Distillate SiO_2, gram	Total SiO_2, gram	Error SiO_2, mg.
1	.....	..	0.1805	0.177	..	0.114	0.114	+114
2	.....	..	0.1822	0.180	..	0.116	0.116	+116
3	Quartz	0.2240	0.1735	0.169	0.2221	0.118	0.3401	+116
4	Silicate solution	0.0802	0.0942	0.092	0.0299	0.0614	0.0913	+11.1
5	Silicate solution	0.1604	0.0917	0.082	0.1151	0.0459	0.1610	+0.6
6	Silicate solution	0.2672	0.1885	0.187	0.1407	0.1264	0.2671	−0.1
7	Silicate solution	0.2672	0.1194	0.114	0.2002	0.0692	0.2694	+2.2
8	Silicate solution	0.2672	0.0904	0.082	0.2204	0.0475	0.2679	+0.7
9	Silicate solution	0.4008	0.0926	..	0.3640	0.0380	0.4020	+1.2
10	Silicate solution	0.4104	0.0926	0.085	0.3750	0.0371	0.4121	+1.7

[a] Single distillation, accurate titration.

Table II. Determination of Silica in Phosphate Rock and Phosphoric Acid by Distillation[a]

	Taken				Found				
No.	Sample	Amount, grams	Form of added silicon	SiO_2 added, gram	Flask SiO_2, gram	Distillate SiO_2, gram	Total less added SiO_2, gram	SiO_2, %	F, %
1	NPR 1[b]	5.005	Quartz	0.1086	0.2352	0.0820	0.2086	4.17	2.43
2	NPR 1[b]	5.002	.....	..	0.1318	0.0718	0.2036	4.07	2.31
3	NPR 1[b]	5.304	Quartz	0.1964	0.3426	0.0785	0.2247	4.24	2.29
4	NPR 1[b]	5.001	Silicate solution	0.2646	0.4154	0.0578	0.2086	4.17	2.09
5	NPR 1[b]	5.001	Silicate solution	0.2646	0.4078	0.0640	0.2072	4.14	2.09
6	NPR 2[b]	5.000	.....	..	0.0135	0.0888	0.1023	2.05	2.67
7	NPR 2[b]	4.999	Quartz	0.2000	0.2077	0.0880	0.0957	1.91	2.72
8	NPR 2[b]	5.003	Silicate solution	0.2843	0.2692	0.0708	0.0557	1.11	2.29
9	NPR 2[b]	5.000	Silicate solution	0.2843	0.2702	0.0717	0.0576	1.15	2.27
10	NPR 3[b]	4.49	Silicate solution	0.2646	0.3208	0.0764	0.1326	2.95	2.77
11	NPR 3[b]	5.19	Silicate solution	0.2646	0.3297	0.0863	0.1514	2.92	2.77
12	Crude phosphoric acid	32.34	Quartz	0.2000	0.2019	0.1215	0.1234	0.382	0.504
13	Crude phosphoric acid	32.34	Silicate solution	0.2646	0.2460	0.1144	0.0958	0.296	0.491
14	Crude phosphoric acid	32.34	Silicate solution	0.2646	0.2504	0.1132	0.0990	0.306	0.491
15	NBS 56b[c]	2.501	.....	..	0.2089	0.0459	0.2548	10.19	3.50
16	NBS 56b[c]	2.524	Silicate solution	0.2672	0.4859	0.0414	0.2601	10.31	3.38
17	NBS 56b[c]	2.474	Silicate solution	0.2672	0.4799	0.0410	0.2537	10.25	3.46
18	NBS 56b[c]	2.521	Silicate solution	0.4104	0.6365	0.0289	0.2550	10.12	3.07
19	NBS 56b[c]	2.565	Silicate solution	0.4008	0.6273	0.0292	0.2557	9.97	3.39
20	NBS 56b[c]	2.504	Silicate solution	0.5344	0.7557	0.0273	0.2486	9.93	3.20

[a] Single distillation, accurate titration.
[b] Different samples of Negev, Israel, phosphate rock.
[c] U. S. National Bureau of Standards, No. 56b (Tennessee brown rock phosphate). Certified analysis; 10.1% silica, 3.4% fluorine.

When 200 to 400 mg. of silica are present, attack on the glassware does not amount to more than 1 or 2 mg.

As the amount of silica added is increased, the recovery of fluorine in the distillate decreases. When the sides of the apparatus were washed down halfway through the distillation, the recovery of fluorine was better but still not complete. It may be assumed that part of the material which gets thrown onto the walls entraps fluorine and does not take part in the reaction. However, the results for silica were not substantially affected by the recovery of fluorine. The explanation offered is that the fluorine remaining attached to the silica is liberated as hydrogen fluoride by a hydrolysis reaction during the ignition of the silica. From a consideration of the equilibrium

$$SiF_4 + 4H_2O \leftrightharpoons Si(OH)_4 + 4HF$$

which has been investigated by Lenfesty and coworkers (*7*) it follows that as the concentration of fluoride is reduced, the ratio of silicon tetrafluoride to hydrogen fluoride will also drop. This suggests that, in the above analyses, if the fluorine remaining in the silica residue is liberated slowly and with free access of moist air, it escapes mainly as hydrogen fluoride. This would not introduce any error into the results. That the fluorine in fact escapes during the ignition was confirmed by fusing such an ignited residue with alkali and distilling from perchloric acid. Practically no fluorine was recovered.

Table III. Determination of Silicon and Fluorine[a] in Tennessee Standard Phosphate Rock[b]

	1	2
Weight of sample, grams	2.5046	2.5069
Silica added, gram	0.2735	0.2735
1st distillation		
Silica in distillate, gram	0.0345	0.0338
Fluorine in distillate, gram	0.0798	0.0850
2nd distillation		
Silica in distillate, gram	None	None
Silica in flask, gram	0.4939	0.4926
Fluorine in distillate, gram	0.0058	0.0029
Silica found, %	10.18	10.09
Fluorine found, %	3.42	3.51

[a] Accurate procedure.
[b] National Bureau of Standards, No. 56b. Certified analysis. 10.1% silica, 3.4% fluorine.

In the experiments reported in Tables I and II the fluorine-to-silicon atomic ratio in the distillate varied between 4 and 10, the higher values, in general, corresponding with lower concentrations of fluorine in the distilling flask. In the second distillation reported in Table III, where the fluorine concentration was much lower, silicon could not be detected in the distillate. This effect is in agreement with the results reported by Shell and Craig (*11*). This means that the fluorine, when present in solution is high concentration, distills largely as silicon tetrafluoride, and when present in low concentration it distills mainly as hydrogen fluoride.

In Table II, sample NPR 1 gave practically the same results whether or not sodium silicate was added, and so did the standard Tennessee rock phosphate; on the other hand, sample NPR 2 gave a very high result in absence of added silicate. This indicates the presence of sufficient freely available silica in the first two cases and of a deficiency in the other case. Total silica content is no indication of the amount freely available to hydrofluoric acid, as shown by quartz being almost entirely immune to attack under the experimental conditions.

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Miniature Fluorescent X-Ray Spectrograph

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A simple, inexpensive x-ray spectrograph with no moving parts and with provision for recording the spectra from two specimens simultaneously, side by side, on photographic film is described. The instrument is about the size of a small x-ray powder diffraction camera and is used on ordinary x-ray diffraction-type equipment. Its main use is in qualitative and semiquantitative analysis, and it covers the same range of elements as the usual Geiger-counter, fluorescent, x-ray spectrometers. With exposure times of 0.5 to 1 hour, concentrations of 2% manganese in an iron matrix and of less than 0.5% manganese in an aluminum matrix may be detected. Resolution is good enough to separate manganese-$K\alpha$ radiation at 2.10 A. from chromium-$K\beta$ radiation at 2.08 A.

FLUORESCENT x-ray spectroscopy has become widely recognized in the past five years as a very powerful analytical tool. This recognition is based primarily on the development of Geiger-counter, x-ray spectrometers of great sensitivity, high resolution, and ease of automation. There remains, however, a large area of qualitative and semiquantitative chemical analysis wherein x-ray spectroscopy is not applied because the problems do not warrant the rather elaborate and expensive Geiger-counter equipment, and unfortunately, no simple, inexpensive spectrographs have been available. In an attempt to satisfy the requirements of this large middle ground of x-ray spectroscopy, the present simple spectrograph has been constructed. It records the x-ray spectra on photographic film and is used on ordinary x-ray diffraction apparatus in much

the same manner as powder diffraction cameras and with comparable exposure times of 0.5 to 1 hour.

INSTRUMENTATION

The principle of the present spectrograph is shown schematically in the plan view of Figure 1. Primary x-rays strike the specimen in the usual fashion and excite fluorescent x-radiation. It is the crystal arrangement which distinguishes this instrument and permits the whole spectrum to be recorded simultaneously without any moving parts. In this figure, diffraction is by planes which are parallel to the narrow edge of the crystal so that only a narrow bundle of radiation of each wave length is passed on to the photographic film. Thus, the diffracting region limits resolution and eliminates the need for any collimating device. This narrow diffracting region is obtained either by using a very thin crystal slab (thicknesses of 0.004 inch are easily obtained with the alkali halides) or by moving the limiting edge in close to act as a half slit so that only radiation diffracted from a narrow region of a thicker crystal is allowed to pass on to the photographic film. The shield next to the crystal prevents stray radiation from being transmitted through the crystal.

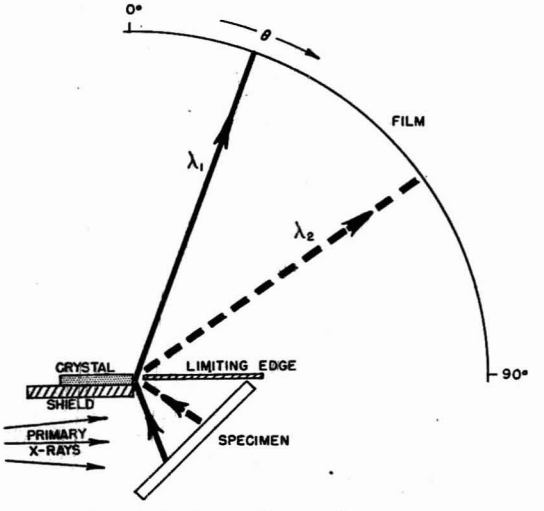

Figure 1. Principle of simple x-ray spectrograph with no moving parts

In the figure fluorescent radiation of wave length, λ_1, emerging in a parallel bundle from only one particular small area on the specimen is diffracted by the crystal while radiation of wave length, λ_2, must emerge from a different area; this means the the specimens must be homogeneous if semiquantitative analyses are to be performed. With a fixed crystal, the angular measure along the film is in terms of θ rather than 2 θ, so that a 90° arc covers the complete diffraction region.

Actually, the instrument as shown in Figure 2 is two spectrographs side by side—that is, two units such as shown in Figure 1 with two specimens and two crystals yielding two spectra simultaneously. In Figure 2, the housing, *A*, has window, *B*, for the primary beam (may be covered with Mylar) and a pumping tube, *C*, for evacuation or admitting helium; *D* is a light-tight shutter for the window, *B*, so that the film may be loaded away from the x-ray tube. The specimens are mounted at *E* at about 45° with respect to the primary beam; this angle is not critical and might be changed to a smaller or greater angle to favor either the longer or shorter wave lengths. Specimen size and distance from the crystal are not important as long as the specimen covers the desired angular range with respect to the crystal. The crystal is shown at *F* but only the top edge is visible in the photograph. The arc, *G*, for holding the film has a radius of 57.3 mm., so that 1 mm. on the film represents 1 degree θ. The separator, *H*, is used to keep the two spectra from overlapping; a similar separator is used between the two specimens but is not shown in the figure. Thus with both crystals alike, a standard and unknown specimen may be compared, or two crystals of different spacing may be used with a single, large specimen to cover a greater range in wave length.

EXPERIMENTAL RESULTS

Both qualitative and semiquantitative results have been obtained for a range of elements, and two examples are shown in Figures 3 and 4. Figure 3 shows typical spectra for a series of salts containing a range of elements from chromium (24) to lead (82). A standard, tungsten-target, x-ray diffraction tube was operated at 50 kv. and 20 ma. with exposures between 0.5 and 1 hour. In making satisfactory prints for publication, the contrast on the original films was increased and therefore has broadened the darker lines considerably. The left edge of each spectra represents 0 degree θ; the high θ angle ends on the right have been removed above about 50 degrees θ. These spectra were taken two at a time with a lithium fluoride analyzing crystal for each specimen. In spectra 1 and 2, the adjacent elements of copper and nickel are easily distinguished. In spectrum 5, chromium shows up even in the presence of lead. Spectrum 6, which is a mixture of copper, nickel, iron, and chromium salts, shows all the elements resolved.

Table I. Composition of Nickel-Chromium Steels

Specimen	Composition, % Cr	Ni	Mn
1	23.7	13.6	0.21
2	18.2	8.6	0.53
3	9.1	0.57	2.07
4	5.5	6.8	1.57
5	2.95	25.7	0.23

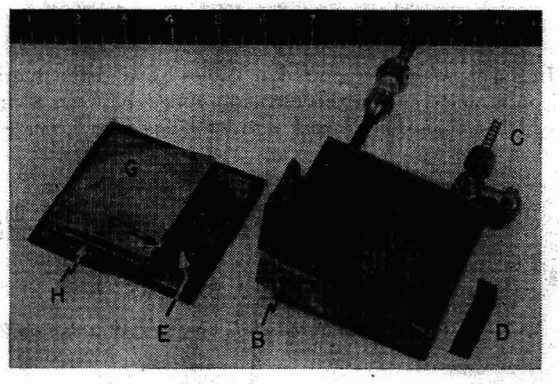

Figure 2. X-ray spectrograph opened for loading

A. Housing
B. Window for primary x-rays
C. Tube for evacuation or admitting helium
D. Light-tight shutter for window
E. Specimens
F. Crystal (only top edge can be seen)
G. Arc for holding photographic film
H. Separators

Lithium fluoride is a convenient crystal for wave lengths as short as 0.50 A. (silver $K\alpha$). For the long wave lengths from elements of atomic number lower than titanium (22), it is necessary to use a crystal with an interplanar spacing greater than

that of lithium fluoride (2.01 A.), and it is desirable to fill the spectrograph with helium to reduce attenuation of the radiation. Ammonium dihydrogen phosphate with an interplanar spacing of 3.74 A. is one example of a satisfactory analyzing crystal for elements from silicon (14) to chromium (24). For instance, the potassium and chlorine lines from potassium chloride salt are strong with a 1.5-hour exposure. For elements of atomic number lower than silicon (14) no completely satisfactory crystals have yet been found.

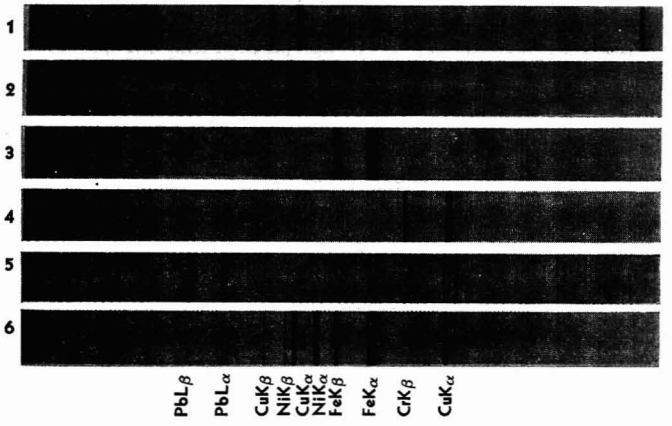

Figure 3. Spectra of a series of powder samples

1. $CuSO_4$
2. $NiSO_4$
3. Fe_2O_3
4. $CrCl_3$
5. $PbCrO_4$
6. Mixture of 1, 2, 3, and 4

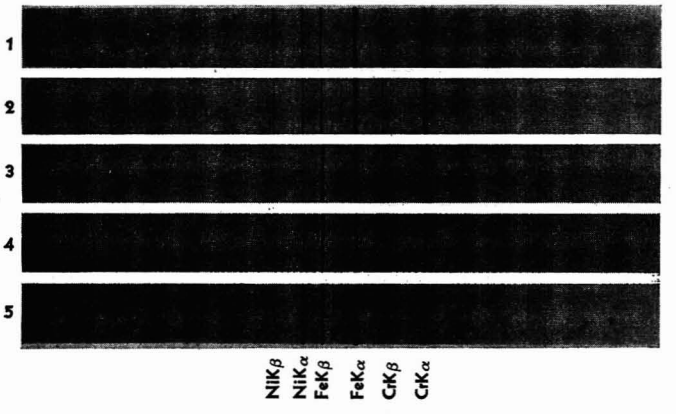

Figure 4. Spectra of nickel-chromium steels

Composition in Table I

Figure 4 shows typical spectra from a series of alloy steels with the composition listed in Table I. X-ray tube operating conditions were the same as for the salts in Figure 3. In the table, the chromium ranged from 2.95 to 23.7%; the nickel ranged from 0.57 to 25.7%; manganese ranged from 0.21 to 2.07%. In Figure 4, the chromium content decreases steadily from spectra 1 through 5 and this is apparent from the appearance of the chromium-$K\alpha$ lines. A semiquantitative analysis for nickel and chromium may be made from Figure 4 either visually or by microphotometering the films. In Figure 5, the chromium and nickel intensities (microphotometered line densities) are plotted against weight per cent. No attempt was made to correct the intensity from one element for the variation in concentration of the other elements, but generally speaking the points appear to fall on a smooth curve. The lines from the major constituents in Figure 4 are so strong that it is difficult visually to judge the intensity of the weaker lines relative to them. This may be overcome by masking the film off in parallel strips extending over the entire θ range. One third of the width of the film on each side of the spectrograph was covered with 0.002 inch of aluminum foil and the next one third with 0.001 inch of aluminum foil. This resulted effectively in three separate exposures on each side due to the partial absorption of the aluminum. Strong lines, after passing through the 0.002 in aluminum, were reduced enough for visual comparison with weaker lines striking the part of film left uncovered.

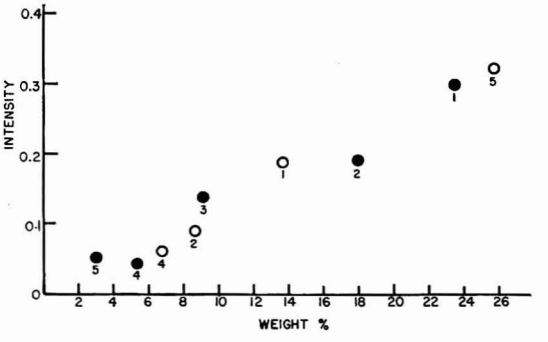

Figure 5. Uncorrected chromium and nickel intensities (microphotometered line densities) from spectra of Figure 4

●. Cr
○. Ni
Numbers are specimen numbers of Table I

Figure 4 illustrates two other points worth mentioning. In spectra 3 the line from manganese $K\alpha$ at 2.10 A. is visible immediately to the right of the chromium-$K\beta$ line at 2.08 A. With the lithium fluoride crystal, the separation of these lines is only 0.3 degree θ representing resolution comparable to that obtained with much more elaborate instruments. The minimum concentration detectable in these specimens was 1.57% manganese in spectra 4 (it was discernible on the original film but has been lost in reproduction). Of course a much lower concentration is detectable in a light matrix. For example, in an aluminum alloy, lines from 0.5% iron and 0.81% manganese were strong with a 45-minute exposure on a copper target x-ray tube.

SUMMARY

The spectrograph described is a simple, inexpensive tool for analyses which do not require the ultimate sensitivity or greater accuracy of a Geiger-counter spectrometer. It is used with ordinary diffraction equipment, so that elemental analysis may be performed at the same time as powder diffraction. By photographing two spectra simultaneously, one can either compare an unknown with a standard or cover a greater range of wave length. Since only a small slab of crystal is required (in fact the crystal can be in the form of a needle mounted parallel to the limiting edge), the choice of analyzing crystal material is much wider than with conventional Geiger-counter spectrometers.

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Determination of Traces of Boron in Silicon, Germanium, and Germanium Dioxide

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In semiconductor research and development there is a need for methods of chemical determination of the Group III and Group V metals appearing as impurities in the semiconductor materials used. A method for the determination of 0.1 to 1 p.p.m. of boron in silicon, germanium, and germanium dioxide is described, in which the sample is dissolved in aqueous sodium hydroxide solution, sodium silicate or germanate is removed by precipitation with methanol, and boron is isolated by distillation as methyl borate and determined photometrically by the curcumin method.

THE present work represents a continuation of the program in progress in this laboratory, for the development of photometric methods for the determination of Group III and Group V metals appearing as impurities in semiconductor materials (*2*). The method described provides for the determination of 0.1 to 1 p.p.m. of boron in silicon, germanium, and germanium dioxide. In order to determine such small quantities of boron it is necessary to use the most sensitive photometric method available—the curcumin method. Because of the lack of specificity of this method, the boron must be isolated by methanol distillation before its determination is attempted. Before such a distillation can be made, however, it is necessary to remove most of the silicon or germanium; otherwise severe bumping will occur as a result of the presence of precipitated salts of the metals.

SEPARATION OF BORON FROM SILICON

Dissolution of metallic silicon in nitric and hydrofluoric acids is not feasible in the present instance because of the loss of boron by volatilization. Fusion with sodium carbonate is not recommended because of the danger of damage to the platinum crucible. Fortunately, silicon, in the powdered form, dissolves in aqueous sodium hydroxide solution, and this method of dissolution has been used in the present case. The dissolution process is rather slow and tedious even when the powder has been finely divided. Attempts to accelerate this by the use of hydrogen peroxide were not successful.

The tendency for boron to accompany silicon makes the analytical chemical separation of these two elements very difficult. Many unsuccessful attempts were made before a satisfactory separation was found. While investigating the possibilities of using ion exchange techniques it was noted that under certain conditions it was possible to precipitate sodium silicate in readily filterable form from aqueous sodium hydroxide solution by the addition of excess methanol. It seemed probable that this could be made the basis of a method for separation of the great bulk of the silicon before final isolation by methanol distillation. Further experiments proved this to be true. If the ratio of methanol to water is kept high and the proper amount of sodium hydroxide is used, most of the sodium silicate precipitates in filterable form without appreciable coprecipitation of the sodium borate. The sodium silicate precipitate is colloidal in nature if too little sodium hydroxide is used. On the other hand, the use of too much alkali is objectionable because this would lead, subsequently, to the formation of large amounts of salts, which would necessitate filtration to prevent bumping in the distillation step. Enough water must be present to keep the sodium borate dissolved, but if too much is present the precipitation of the silicate will be less complete and more sodium hydroxide will be required to coagulate the precipitate. About 15% of the boron present is coprecipitated with the silicate. In order to obtain satisfactory recoveries it is necessary, therefore, to resort to a double precipitation. If the two filtrates are combined previous to the distillation, the recovery of boron is only slightly better than that obtained with a single precipitation; if each filtrate is analyzed independently, the recoveries are satisfactory. When a solution containing sodium borate and sodium silicate is acidified, boron is lost by adsorption or occlusion in the silicic acid if more than a few milligrams of the latter are present. Hence it is probable that the low results obtained when the filtrates are combined results from incomplete recovery of the boron during the distillation step as a result of the high concentration of silicic acid and sodium sulfate present.

SEPARATION OF BORON FROM GERMANIUM OR GERMANIUM DIOXIDE

Boron can be separated from germanium in much the same manner as from silicon. Germanium dioxide is readily soluble in aqueous sodium hydroxide solution, but in order to dissolve germanium metal, it is necessary to add hydrogen peroxide. The commercially available 30% hydrogen peroxide used in the development of the method—i.e., Merck's Superoxol—was found to contain more boron than could be tolerated. As hydrogen peroxide is only slightly ionized, it appeared probable that the borate present could be removed on an anion exchange resin. Attempts to do this were, however, not successful. The resin used—i.e., analytical grade of Amberlite IRA-400 (OH) modified amine-type synthetic anion exchange resin supplied by Rohm and Haas Co.—was attacked by the peroxide with the liberation of heat and a considerable amount of oxygen was evolved in the form of tiny bubbles. Under these circumstances little or no boron was taken up by the resin. Fortunately, it was found that the boron could be readily removed by the use of a mixed cation-anion exchange resin (analytical grade of Amberlite MB-1 sulfonic acid–amine type of mixed synthetic cation and anion exchange resins supplied by Rohm and Haas Co.). When this mixed resin is used, little or no reaction with the peroxide occurs, no gassing is seen, and both cations and anions are removed rapidly. The failure of the anion exchange resin to remove the boron can probably be explained by the fact that the bubbles of oxygen, liberated owing to the extreme alkalinity of the resin as supplied, prevent the boron from making contact with the resin. With the more nearly neutral mixed resin, decomposition of the peroxide does not occur and hence there is no barrier between the boron and the anion exchange resin.

In contrast to the experience with silicon, it was found that a single methanol separation suffices to separate the boron quantitatively from the bulk of the germanium. Apparently boron does not tend to accompany germanium as tenaciously as it does silicon. Fairly large quantities of germanium can be tolerated during the methanol distillation.

It is probable that the methanol method can be used for the separation of silicate or germanate from trace metals other than boron.

ISOLATION OF BORON BY DISTILLATION

The apparatus shown in Figure 1 has been used for the distillation of boron as methyl borate. With this equipment it is

possible to recover only about 90% of the boron evolved in the distillation. Apparently some boron is lost via the pressure regulator tube, by failure to be retained in the sodium hydroxide solution, or both. In any event it was felt that the simplicity of the apparatus justified its use.

Distillation of traces of boron is quantitative from a mixture of 1 ml. of distilled water plus 25 ml. of methanol, but becomes increasingly less quantitative as the amount of water increases. Thus, only about half the boron is removed by distillation from a mixture of 4 ml. of distilled water and 25 ml. of methanol. The presence of sulfuric acid in the flask at the time of distillation tends to prevent complete distillation. Thus the removal of boron from a solution of 2 ml. of sulfuric acid (1 + 1) plus 25 ml. of methanol is only 90% complete. On the other hand, in the method in question it has been found more convenient to add sufficient sulfuric acid to keep the sodium salts in solution than to have to make a filtration previous to the distillation. Moreover, losses of boron due to coprecipitation with sodium sulfate and silicic acid are reduced if the filtration step is eliminated. The combined loss of boron due to incomplete distillation and incomplete recovery in the receiver dish is less than 20%. For this reason it is permissible to compensate for it by including the distillation step in the preparation of the calibration curve used in the photometric determination of the boron.

Care must be taken to prevent contamination of the boron distillate with silicate or germanate, as the former causes low results and the latter high results for boron. In practice it is best to reserve one or two platinum crucibles to be used only as receivers in the boron distillation.

PHOTOMETRIC DETERMINATION OF BORON

The only photometric method possessing sufficient sensitivity for the boron determination is the curcumin method (*1*). This method is, however, difficult to use, because very close control of many variables is required if reproducible values are to be obtained. Even with the best technique the results are not so good as one would like them to be. In practice it is probable that the limitations of the photometric method will prevent accuracy within about ±10% for the over-all method.

When the photometric method is used, concentrations and volumes of reagents, times, temperatures, procedure for evaporation, and method of dissolving the colored boron compound—in fact, all the manipulations involved in the photometric estimation—must be closely duplicated. Aside from the need to control the relative and absolute quantities of reagents from a purely chemical standpoint, it is necessary to control all chemical and physical factors that influence the rate of evaporation of the solution during the color development. Among such factors are constancy of the temperature of the water bath, shape, smoothness, and thickness of the platinum dish, temperature of the room, air currents in the vicinity of the bath, and precipitation of salts during the evaporation. If salts precipitate readily, there will be available a large area of wetted surface and the evaporation will be rapid. On the other hand, if little or no sodium chloride, sodium oxalate, or oxalic acid precipitates during the initial stages of the evaporation, the time required to take the sample to dryness will be increased and the resulting color will be more intense.

It is best to prepare fresh curcumin and sodium hydroxide solutions each day as required. Upon aging of either of the reagent solutions, the color produced with a given amount of boron decreases. It is probable, in the case of the sodium hydroxide, that the decrease in color is caused by some material extracted from the polyethylene by the alkali.

APPARATUS

Forged Tool Steel Plattner Mortar and Pestle. It is essential that the mortar and pestle used be sufficiently smooth and hard to prevent excessive contamination of the sample with iron.

Platinum Sieve. A circular piece of 150-mesh platinum wire screen 2.5 inches in diameter, supported in a Plexiglas frame.

Platinum Dishes, 100-ml.

Polyethylene Ware. A 60° funnel and 125-ml. and 500-ml. bottles with screw caps.

Polyethylene Police Rods, supplied by the New York Laboratory Supply Co., Inc. They consist of 6-inch metal rods hermetically sealed in polyethylene tubing (1/4 inch in outside diameter). At each end the tubing has been flattened into a thin fan-shaped paddle about 1/16 inch thick and 1/2 inch square.

Polystyrene Graduates and Dropping Pipets, supplied by Arthur H. Thomas Co., Inc. The graduates have 10-ml. capacity and are graduated in 0.2-ml. subdivisions. The dropping pipets are supplied with rubber bulbs.

Conical Flasks (low-boron). These are 300-ml. flasks made from Corning Brand alkali-resistant glass No. 7280.

Wash Bottles with Hand-Operated Rubber Pressure Bulbs. These are of 500-ml. capacity and are obtainable from the K & K Glassware Co., Bloomfield, N. J.

Acid-Washed Filter Paper. In order to ensure that the filter paper used shall be virtually free of boron, seat a 9-cm. No. 41 Whatman filter paper on a 60° glass funnel, fill to overflowing with hot hydrochloric acid (1 + 9), and allow to drain through the paper. Repeat this process until 100 ml. of the diluted acid has been used. Then wash repeatedly with distilled water until the washings no longer turn red when treated with a few drops of methyl red indicator solution. (If the acid is not washed out, sodium silicate or germanate may be dissolved during the subsequent filtrations. It is desirable to avoid contaminating the filtrate with chloride, in the analyses of germanium and germanium dioxide, for fear of contaminating the subsequent boron distillate with germanium tetrachloride.) Wash the paper three times with a fine stream of redistilled methanol from a wash bottle to remove the water. Cover the funnel with a watch glass and reserve until needed.

Quartz Distillation Apparatus. This consists of a clear quartz 100-ml. conical flask with a quartz standard-taper cap which has a side arm and a pressure-regulator tube that extends to within 1 mm. of the bottom of the flask. As a precaution against breakage, the flask is fitted with a heavy lead collar.

Spectrophotometer. A Beckman Model B spectrophotometer with 5-cm. absorption cells and the blue-sensitive phototube was used in the development of the method.

REAGENTS

Distilled Water. The distilled water used in the preparation of the reagents and in the procedure must be low in boron. In the development of the method ordinary distilled water was passed through a mixture of cation exchange and anion exchange resins before use.

Standard Boron Solution (0.5 γ of boron per ml.). Dissolve 0.2860 gram of reagent grade boric acid in water and dilute to 1 liter in a volumetric flask. Transfer 5.0 ml. of this solution to a 500-ml. volumetric flask and dilute to the mark. Store in a polyethylene bottle.

Sodium Hydroxide Solution (5%). Transfer 5.0 grams of reagent grade pellet sodium hydroxide to a clean dry polyethylene bottle and add 100.0 ml. of distilled water from a pipet. Swirl to dissolve the pellets. Prepare fresh daily.

Oxalic acid (low-boron). Recrystallize reagent grade oxalic acid from distilled water and dry overnight in a vacuum desiccator.

Ethanol (low-boron). Transfer 350 ml. of absolute ethanol plus 2 ml. of sodium hydroxide solution (5%) plus a few crystals of 20- to 30-mesh silicon carbide to a 500-ml. Pyrex flask. Connect by means of a low-boron glass 24/40 standard-taper joint to a water cooled low-boron glass condenser (Corning Brand alkali-resistant glass No. 7280). Start the distillation, discard a few milliliters of the distillate, and then catch the next 300 ml. in a low-boron glass conical flask. Cover with a watch glass. Ethanol or methanol should not be stored in polyethylene bottles, as these solvents dissolve out small amounts of a waxlike substance.

Methanol (low-boron). Proceed as for the preparation of low-boron ethanol.

Curcumin–Oxalic Acid Solution. Transfer 0.0050 gram of Eastman's crystalline curcumin plus 0.500 gram of recrystallized oxalic acid to a clean 150-ml. beaker. Pipet 50.0 ml. of low-boron ethanol to the beaker while swirling to dissolve the oxalic acid and curcumin. When solution is complete, transfer to a 50-ml. buret. Prepare fresh daily.

Sulfuric Acid (low-boron). Transfer 2 ml. of sulfuric acid to a 100-ml. quartz conical flask (see Figure 1). Add a few crystals of 20- to 30-mesh silicon carbide and 20 ml. of low-boron methanol. Evaporate without cover on a low temperature hot plate—i.e.,

170° to 180° C.—until white fumes appear. Continue heating until the foaming of the solution in the flask ceases. Avoid prolonged heating; otherwise charring of the remaining methanol will occur. Cool and pour the acid into a 125-ml. polyethylene bottle. Repeat this process several times until a sufficient supply of acid has been accumulated. Mix well by swirling.

Hydrogen Peroxide (low-boron). Transfer 5 grams of Amberlite MB-1—i.e., analytical grade of sulfonic acid-amine type of mixed synthetic cation and anion exchange resins supplied by Rohm and Haas Co.—to each of two 250-ml. beakers. Add 25 ml. of 30% hydrogen peroxide to one of the beakers and swirl vigorously for 2 minutes. Filter immediately through a dry, acid-washed 9-cm. No. 41 Whatman filter paper to a small brown glass bottle. Do not wash. Repeat this process immediately with the second beaker, filtering through the same paper and collecting in the same brown bottle. Swirl to mix the purified hydrogen peroxide solution. As a precaution against fire or explosion, wash the resin and filter paper well to remove the peroxide before discarding.

PREPARATION OF CALIBRATION CURVE

Carry through each of the following standards individually. Transfer 0, 0.25, 0.50, 0.75, and 1.00 ml. of standard boron solution (0.5 γ of boron per ml.) to a clean 100-ml. quartz distillation flask (see Figure 1). Add sufficient distilled water from a pipet to make a total of 1-ml. volume in each case. Add 1.0 ml. of low-boron sulfuric acid from a pipet or graduate. Add 25 ml. of redistilled methanol and three or four silicon carbide crystals. Put on the lead collar and cap with the distillation head.

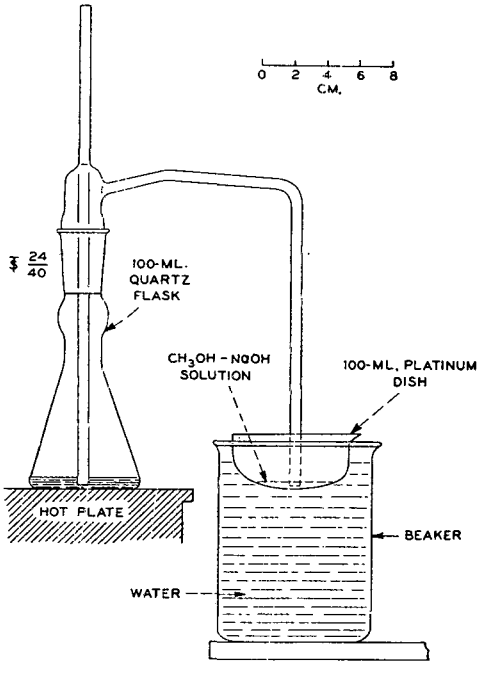

Figure 1. Apparatus

Transfer 2.0 ml. of 5% sodium hydroxide solution by means of a polystyrene graduate or pipet, plus about 8 ml. of redistilled methanol, to a 100-ml. platinum dish. Float the dish on water in a 600-ml. beaker as shown in Figure 1, and adjust the water level so that when the distillation flask is placed on the hot plate the side arm dips to within 1 mm. of the bottom of the dish. With the surface temperature of the plate adjusted to about 170° C., heat the solution to boiling. As the distillation proceeds it may be necessary to raise the level of the water in the beaker to prevent the solution from being thrown out of the dish. Finally, when the distillation is complete, as indicated by the fact that the liquid column in the pressure regulator tube breaks or when gas bubbles no longer emerge from the side arm in the dish, remove the flask. Do not attempt to distill over the water that remains in the flask after the removal of the methanol, as no more boron will be recovered.

Float the platinum dish in 500 ml. of hot water in a 600-ml. beaker which is heated on the same 170° C. hot plate. In order to speed up the evaporation of the methanol it is convenient to use a gentle jet of air, which passes from the laboratory low pressure air system through a little absorbent cotton in a glass nozzle which is aimed at the surface of the solution in the dish. When distillation of the methanol is complete and the alkali residue is dry, remove from the water bath and cool.

Float the dish on cool water in a beaker and add 1.00 ml. of hydrochloric acid (1 + 1) from a buret. Swirl to dissolve most of the alkali. After the solution has cooled, remove the dish from the water bath and swirl to wet every trace of alkali on the walls of the dish. Add 2.00 ml. of curcumin–oxalic acid solution from a buret. Swirl to mix well. Float the dish in 55° C. water in a 600-ml. beaker which is resting on a low temperature hot plate in a draft-free location. Avoid direct sunlight. Maintain the water at a temperature of 55° ± 1° C. and adjust its level so that the top of the platinum dish is level with the top of the beaker. Allow the dish to remain until all the liquid therein has evaporated.

When the last "gray" patches have disappeared from the orange colored residue and the odor of hydrochloric acid can no longer be detected, remove the dish at once from the bath. Add 15.0 ml. of ethanol from a pipet and swirl vigorously for 1 or 2 minutes to extract the orange-red colored material from the oxalic acid–sodium chloride residue. If necessary, tilt the dish to wet any residue that has been deposited near its rim. Do not attempt to loosen the residue from the dish with a stirring rod; otherwise it may become too finely divided. Allow the residue to settle for a moment and then decant a sufficient amount of the ethanol solution to a 5-cm. absorption cell. Insert the cell in the photometer, allow the solution to settle for a minute or so, and then measure photometrically at 540 mμ, using ethanol as the reference sample. Prepare a calibration curve.

DETERMINATION OF BORON IN SILICON

Crush a portion of the silicon sample to be analyzed in a clean, rust-free Plattner mortar into small pieces and then pound vigorously to break the metal into as fine particles as possible. Remove the powder to a 150-mesh platinum sieve and shake to recover that portion of the sample which will pass through the screen. Return the residue to the mortar and repeat the pounding. Alternately pound and sieve the metal until all has been passed through the sieve. Mix well and pass through the sieve a second time to remove any large particles which may have accidentally fallen in.

Transfer 0.100 gram of the 150-mesh silicon metal to a 100-ml. platinum dish. Do not cover. Add 4.0 ml. of 5% sodium hydroxide solution and warm the dish momentarily on a low temperature hot plate to start the dissolution of the sample. Warm gently from time to time to keep the reaction going. Avoid such rapid dissolution that sample is lost by excessive spraying. Finally, when it is safe to do so, place the dish on a 250-ml. beaker about half filled with vigorously boiling water.

Allow the solution in the dish to evaporate to apparent dryness and continue to heat on the steam bath for a few minutes to promote more rapid attack of the sample. Finally wash down the walls of the dish with 3 or 4 ml. of distilled water and again allow to evaporate to dryness. Repeat this process one or more times if necessary. Finally, when most of the sample has been dissolved, add 3 or 4 ml. of distilled water, scrub down the walls of the dish with this water using a polyethylene police rod to loosen the material deposited there by spray, and then wash down the walls and police rod with a fine stream of water from a wash bottle. Remove the police rod. Evaporate the solution to dryness on the steam bath. Then alternately add 3 or 4 ml. of water and allow the solution to evaporate to dryness until all but traces of the sample have dissolved.

Finally evaporate the sample to dryness on the steam bath. Add 5 ml. of distilled water and warm to dissolve the salts. In order to ensure complete solution of the viscous alkaline paste, stir and scrape the bottom of the dish with a pclyethylene police rod. Remove the dish from the steam bath and add, with stirring, 25 ml. of redistilled methanol. Continue to stir and scrape the sides and bottom of the dish with the police rod until the precipitate of sodium silicate and sodium hydroxide coagulates. Filter through an acid-washed 9-cm. No. 41 Whatman filter paper which has been seated in a 60° polyethylene funnel and wetted by a fine stream of redistilled methanol from a wash bottle. (Hold a police rod so that the paddle is resting perpendicularly to the outside edge of the lip of the platinum dish while pouring the

solution from the dish over the flattened portion of the rod. In this way the tendency for the solution to run down the side of the dish can be eliminated.) Collect the filtrate in a 100-ml. platinum dish. Drain the solution from the platinum dish to the filter paper but retain as much of the precipitate as possible in the dish. Do not wash.

Allow the solution on the paper to drain completely to the second platinum dish, but do not wash. Reserve the paper. Replace the dish containing the filtrate by a third 100-ml. platinum dish. Place the dish containing the filtrate in a hot water bath under an air jet and evaporate to dryness. While the above filtration and evaporation are proceeding, transfer 1.0 ml. of 5% sodium hydroxide solution plus 4 ml. of distilled water to the original platinum dish and warm on a steam bath to dissolve the sodium silicate and sodium hydroxide. Stir and rub the bottom of the dish with the reserved police rod to dissolve traces of precipitate from the latter. Then add 25 ml. of redistilled methanol and repeat the filtration through the filter paper reserved from the first precipitation. Completely fill the paper at least once during the filtration. When the filtration is complete, wash the dish and paper once with a fine stream of redistilled methanol from a wash bottle. Discard the paper and precipitate. Evaporate the second filtrate to dryness under an air jet in a hot water bath.

Carry each of the two residues independently through the following procedure: Add 1.0 ml. of distilled water to the dish from a pipet. Tilt and rotate the dish to wet all the precipitate. Warm momentarily on the steam bath to dissolve as much of the precipitate as possible. Add, with stirring, 25 ml. of redistilled methanol followed by 1.0 ml. of low-boron sulfuric acid. Ignore any precipitate of sodium sulfate, as this will usually dissolve subsequently when the solution is heated to boiling. Pour and wash with redistilled methanol into a 100-ml. quartz distillation flask. Add three or four crystals of silicon carbide, put on the lead collar, cap with the distillation head, and then distill and determine the boron as directed for preparation of calibration curve.

Carry a reagent blank through the entire analysis in the following manner: Transfer 3.0 ml. of 5% sodium hydroxide plus 2.0 ml. of distilled water to a platinum dish. Add, with stirring, 25 ml. of redistilled methanol. Filter through an acid-washed paper, evaporate, distill, and determine boron as directed above. Reserve the paper. Repeat the above process using 2.0 ml. of sodium hydroxide plus 3.0 ml. of distilled water and filtering through the paper reserved from the previous filtration.

With the aid of the calibration curve determine the micrograms of boron present in each of the two filtrates obtained from the sample and those obtained from the reagent blank.

DETERMINATION OF BORON IN GERMANIUM

Crush, powder, and sieve the germanium sample in the manner described above. Transfer 0.100 gram of the 150-mesh germanium metal to a 100-ml. platinum dish. Do not cover. Add 3.0 ml. of 5% sodium hydroxide solution and place on a 250-ml. beaker half filled with boiling water.

Transfer 5.0 ml. of low-boron 30% hydrogen peroxide to a 30-ml. beaker. Add about 0.5 ml. of the peroxide, by means of a dropping pipet, to the platinum dish. From time to time, as the foaming ceases, continue to add further 0.5-ml. portions of the peroxide plus small amounts of distilled water to the dish, to dissolve the powdered metal. The volume of solution must not be allowed to go much below 3 ml., otherwise germanium dioxide will precipitate and seriously retard the solution of the sample. Continue the intermittent addition of peroxide to the hot solution in the dish with occasional swirling and washing down of the dish walls with a fine stream of water, until complete dissolution of the sample is obtained. In order to make most efficient use of the peroxide it is best to eject each portion of the peroxide from the dropping pipet onto that area of the solution directly over the submerged mound of undissolved metal. Complete dissolution should result after the addition of about 4 ml. of the peroxide. When dissolution is complete, add the remainder of the 5 ml. of peroxide, wash down the walls of the dish with distilled water, and then allow to remain on the steam bath until all but traces of the water have been expelled.

Wash down the dish walls with 10 ml. of distilled water and swirl and heat for a few minutes on the steam bath to dissolve all the salts. Remove from the bath and cool to room temperature. Add, with stirring, 25 ml. of redistilled methanol. Filter through an acid-washed 9-cm. No. 41 Whatman filter paper on a polyethylene funnel and wash thoroughly with a fine stream of redistilled methanol from a wash bottle. Collect the filtrate and washings in a 100-ml. platinum dish. Discard the paper and precipitate. Evaporate the filtrate to dryness, first in a hot water bath with the aid of an air jet and finally, when most of the methanol is gone, on a steam bath.

Distill and determine the boron as directed above. Appropriate modifications in the procedure for the determination of the reagent blank must be made. (Germanium salts that may have precipitated in the flask toward the end of the distillation can be removed by boiling with a 10% tartaric acid solution.)

DETERMINATION OF BORON IN GERMANIUM DIOXIDE

Transfer 0.200 gram of the powdered sample to a 100-ml. platinum dish. Add 3.0 ml. of 5% sodium hydroxide solution and, without delay, rub the powder against the bottom of the dish with the paddle of a polyethylene police rod until the powder has dissolved completely. Add 7 ml. of distilled water and mix. Add 25 ml. of redistilled methanol and proceed to the filtration, evaporation, distillation, and photometric determination as for germanium.

EXPERIMENTAL

Typical calibration curve data are shown in Table I.

A sample of Du Pont Hyper-Pure silicon was found to contain 0.6 p.p.m. of boron. Several 0.100-gram portions of this metal were dissolved in sodium hydroxide solution, various aliquot portions of standard boron solution were added, and boron was then determined in the mixtures as described in the method (Table II).

Table I. Typical Calibration Curve Data

No.	Boron Added, γ	% Transmittancy
1	0	72.8
2	0.125	52.7
3	0.25	36.0
4	0.375	26.3
5	0.50	18.6

Table II. Determination of Boron

No.	Boron Added, γ	Total Boron Found, γ	Boron Recovered, γ
	Determination in Silica		
1	0	0.098	...
2	0.125	0.208	0.11
3	0.25	0.350	0.25
4	0.375	0.470	0.37
5	0.50	0.609	0.51
	Determination in Germanium		
1	0	0.078	...
2	0.125	0.198	0.12
3	0.25	0.314	0.24
4	0.375	0.441	0.36
5	0.50	0.549	0.47
	Determination in Germanium Dioxide		
1	0	0.065	...
2	0.125	0.197	0.13
3	0.25	0.304	0.24
4	0.375	0.428	0.36
5	0.50	0.540	0.48

Samples of germanium and germanium dioxide were analyzed and shown to contain no boron. The experiment described above was then repeated, using 0.100-gram portions of germanium and 0.200-gram portions of germanium dioxide. The appropriate sections of the procedure dealing with the analysis of germanium and germanium dioxide were followed. The results obtained are shown in Table II.

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Overcoming the Effect of Manganese Dioxide in Fluoride Determinations

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Both manganese dioxide and chlorides in vegetation samples interfere with the modified Willard-Winter method for the microdetermination of fluoride. The liberation of chlorine during distillation hampers the titration of fluorides by a partial or complete bleaching of the alizarin red indicator. The addition of 3% hydrogen peroxide in the fluoride distillation flask before the start of distillation eliminates this interference. Tests of the reproducibility of fluoride recovery in the presence of 3% hydrogen peroxide as a reductant showed that addition of 3% hydrogen peroxide did not interfere with fluoride recovery or subsequent titration.

MICRODETERMINATIONS of fluoride in plant tissue, such as eucalyptus and citrus leaves, some grasses and root fibers, are often hampered by a fading end point during the titration with thorium nitrate. In some samples there is complete bleaching of the alizarin red S indicator. It has been observed that a correlation exists between the intensity of the blue-green color formed by sodium hydroxide fusion of the limed leaf ash and the amount of interference during titration. The blue-green color is apparently caused by sodium manganate, formed by sodium hydroxide reacting with manganese dioxide. Manganese dioxide in concentrations as high as 0.3% was found in some leaf ash by spectrographic analysis.

After the limed leaf ash has been fused according to the modified Willard-Winter method (*3*), the melt is taken up with water and placed in a distilling flask containing perchloric acid. At this point the sodium manganate undergoes internal oxidation-reduction, forming brown manganese dioxide and sodium permanganate, resulting in a slight purple color to the reaction mixture. As most plant tissue contains soluble chlorides, silver perchlorate is added to the stills before distillation to precipitate the chlorides. However, the formation of the silver chloride precipitate is not complete in this acidic mixture and the permanganate present oxidizes the available chlorides to chlorine which is subsequently carried over into the receiving flask during distillation. The presence of chlorine in the distillate can be demonstrated by its ability to liberate iodine from potassium iodide solutions.

A method for reducing chlorine has been employed by Clifford (*2*) and the Association of Official Agricultural Chemists (*1*) by adding a solution of hydroxylamine hydrochloride to the distillate. The addition of hydroxylamine hydrochloride is normally used to reduce only traces of chlorine in the distillate caused by the slight decomposition of perchloric acid into chlorine and oxygen. However, when larger concentrations of chlorine are present, the author found that the addition of hydroxylamine hydrochloride did not eliminate titration interference. The interference encountered when a larger concentration of chlorine is treated with hydroxylamine hydrochloride characterizes itself by a broadening of the end point and not by bleaching the indicator. The author has never encountered the problem of decomposition of perchloric acid resulting in a partial bleaching of the indicator as reported by Clifford (*2*). Therefore, because only large amounts of chlorine were encountered, the problem was one of preventing chlorine from being distilled.

The immediate problem was to reduce the permanganate in order to prevent chlorine formation. This was solved by reducing the permanganate in the distilling flask with 3% hydrogen peroxide. Swirling of the distilling flask prior to distillation is necessary to ensure complete reaction.

TESTS

The following tests were performed to determine the reproducibility of fluoride recovery in the presence of 3% hydrogen peroxide as a reductant.

Artificial Fluoride Sample. PEROXIDE ADDED, MANGANESE DIOXIDE ABSENT. One gram of lime fused with 7 grams of sodium hydroxide, 50 γ of fluoride (as NaF), 4 drops of 3% hydrogen peroxide, and 1 ml. of 25% silver perchlorate were added to 55 ml. of perchloric acid in a distilling flask. Five hundred milliliters of distillate were collected. The results of several determinations, shown in Table I, are in close accord with other determinations made in the absence of hydrogen peroxide. The hydrogen peroxide added to the distillation flask before distillation did not interfere with fluoride recovery or subsequent titration.

PEROXIDE, CHLORIDE, AND MANGANESE DIOXIDE ADDED. The following determinations were made to establish further proof that hydrogen peroxide does not affect the accuracy of the microdetermination of fluoride. The sodium hydroxide melt which contained 1 gram of lime, 10 mg. of manganese dioxide, and 10 mg. of sodium chloride fused with 7 grams of sodium hydroxide was added to 55 ml. of perchloric acid in a distilling flask, as well as 50 γ of fluoride, sufficient 3% hydrogen peroxide to reduce manganese dioxide and permanganate, and 1 ml. of silver perchlorate. Five hundred milliliters of distillate were collected. Reproducible results are shown in Table II, which are impossible to obtain when hydrogen peroxide is not employed; the indicator is completely bleached in many instances within a few minutes, so that titration is impossible.

Leaf Ash. NATURAL MANGANESE, PEROXIDE ADDED. The following procedure was followed when manganese was found in the leaf samples. Two grams of lime-fixed eucalyptus leaf ash fused with 7 grams of sodium hydroxide, sufficient 3% hydrogen peroxide to reduce manganese dioxide and permanganate, and 1 ml. 25% silver perchlorate were added to 55 ml. of perchloric acid in a distilling flask. Five hundred milliliters of distillate were collected in the subsequent Willard-Winter distillation. Table III shows that a great excess of hydrogen peroxide in the

Table I. Effect of Hydrogen Peroxide on Fluoride Recovery in Artificial Samples in Absence of Manganese Dioxide

Sample No.	% F Recovered	Sample No.	% F Recovered
1	95.2		
2	97.8	5	95.8
3	99.0	6	96.8
4	96.0	7	98.2
		Mean	97.0 ± 1.4[a]

[a] Standard error of mean.

Table II. Effect of Hydrogen Peroxide on Fluoride Recovery in Artificial Samples Containing Manganese Dioxide and Sodium Chloride

Sample No.	% F Recovered	Sample No.	% F Recovered
1	102.0	6	98.8
2	101.2	7	98.6
3	96.0	8	98.4
4	96.4	9	99.2
5	99.6		
		Mean	98.9 ± 1.9[a]

[a] Standard error of mean.

Table III. Effect of Excess Hydrogen Peroxide on Fluoride Recovery with Natural Manganese in One Eucalyptus Leaf Ash

Sample No.	Drops of 3% H_2O_2 in Excess	F Recovered, γ
1	2	323
2	2	318
3	6	314
4	10	318
5	10	312
	Mean	317 ± 5.3[a]

[a] Standard error of mean.

Table IV. Determination of Fluoride in Manganese-Free Citrus Leaves

Sample No.	P.P.M. of F in Leaves: No MnO_2 or 3% H_2O_2 added	P.P.M. of F in Leaves: MnO_2 and 3% H_2O_2 added
1	16.9	18.3
2	21.6	25.3
3	29.2	31.3
4	51.8	55.4
5	38.6	40.5
6	18.1	19.2
7	63.3	63.3
8	49.0	46.0
9	35.5	34.9
10	50.0	53.0
11	58.4	62.1
12	50.9	46.8
	Mean 40.3 ± 10.2[a]	41.4 ± 9.9[a]

[a] Standard error of mean.

distilling flask does not interfere with fluoride recovery or subsequent titration.

MANGANESE-FREE LIMED CITRUS LEAF. This experiment was performed to determine the effect of 3% hydrogen peroxide on the reproducibility of fluoride determinations on leaf samples which did not contain sufficient manganese to cause interference in the titration with thorium nitrate.

The procedure followed was the same as when manganese was present, except that in one case manganese dioxide was added to the fusion and the sample was subsequently reduced in the still with hydrogen peroxide. The results listed in Table IV indicate that the procedure devised for leaf samples causes no appreciable interference with the microdetermination of fluorides.

The method proposed for the elimination of chlorine interference during the titration has proved successful on all types of vegetation tested. The best control measure is observation of the sodium hydroxide melt. When this cooled melt has a definite blue-green color, the reduction procedure using hydrogen peroxide in the distillation flask should be followed.

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Gravimetric Determination of Small Amounts of Tellurium in Sulfur

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The precipitation of small amounts of tellurium by reduction with hydrazine and sulfur dioxide in the presence of hydrochloric acid was studied. Small amounts may be weighed on a microbalance; amounts less than about 0.1 mg. in 5 ml. of solution cannot be precipitated quantitatively. Tellurium can be enriched by coprecipitation as tellurous acid with aluminum hydroxide and thus separated from contaminating soluble salts. A special glass apparatus is used for the determination of tellurium in sulfur.

IN CONNECTION with another investigation the authors were obliged to determine very small amounts of tellurium in sulfur obtained from shales. A large number of methods are described in the literature. An exhaustive survey of the literature on the analytical chemistry of tellurium, up to September 1939, is found in Gmelins Handbuch (*4*).

Two weighing forms have been generally used for the gravimetric determination of tellurium: the element and the dioxide. The methods of the first have been examined, as most suitable for the present problem.

The metallic tellurium is prepared by reduction of acid solutions. Most of the reducing agents proposed earlier are sufficiently effective, although some of them cause undesirable secondary reactions. The simplest and most reliable method is that of Gutbier (*6*), with hydrazine and sulfur dioxide in strong hydrochloric acid solution.

Gravimetric micromethods discussed by Hecht and John (*7*) are based on the work of Clauder (*3*) on a micro scale (*1*, *2*, *7*).

The determinations described below have been performed exclusively by gravimetric methods. The conditions for a good precipitation of tellurium as metal are described by Clauder (*3*). Some handbooks of analytical chemistry, probably as a result of the work of Keller (*8*), suggest that sulfuric acid retains tellurium on reduction, so that the solutions should contain only small amounts of this acid. The present problem is to examine methods suitable for concentrating tellurium present in very dilute solution, find the lowest concentration for good accuracy, and apply the experiences to the determination.

EXPERIMENTAL

Tellurium solutions were prepared from purest tellurous oxide, which was dissolved in potassium hydroxide and diluted to a suitable concentration. Tellurium was precipitated as metal chiefly in accordance with the instructions of Clauder (*3*).

To the tellurium solution were added solutions of 10*N* hydrochloric acid, hydrazine hydrochloride, and sulfur dioxide, so that the resulting concentrations were, respectively, 7 to 8 grams of hydrochloric acid, 1 to 1.5 grams of hydrazine hydrochloride, and 1 to 3 grams of sulfur dioxide per 100 ml. After gradual heating to the boiling point, the reduction mixture was boiled cautiously for a few minutes and then heated on the steam bath for about 1 hour. Small amounts (2 mg. or less) were collected by filtration through a microplatinum filter crucible or a glass filter rod in a glass microbeaker. Larger amounts were collected in a macro platinum filter crucible. The precipitated tellurium was washed

with cold water and with ethyl alcohol, dried at 105° C., and weighed.

Hecht and John (7) found that a good determination can be performed when the concentration of tellurium is 0.2 mg. or more in 5 ml. There are some difficulties involved in accurately weighing very small amounts of tellurium (less than 0.1 mg.). The present experiments, however, have shown that determinations can be performed to about 0.1 mg. in 5 ml. of solution with sufficiently good accuracy. The accuracy is obviously more dependent on the correct weighing of the precipitate than on the performance of the precipitation reactions.

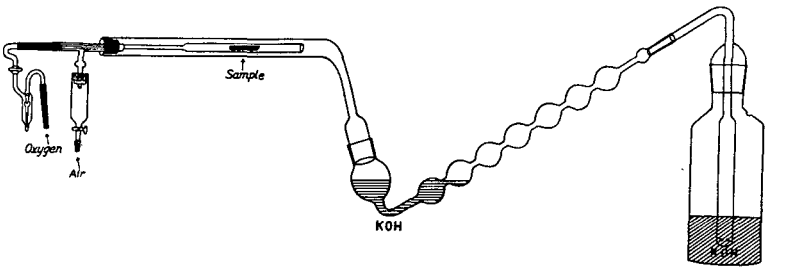

Figure 1. Apparatus for burning of sulfur

CONCENTRATION OF TELLURIUM IN VERY DILUTE SOLUTIONS

As it is difficult to precipitate tellurium quantitatively from solutions containing less than 0.5 mg. in 100 ml., it is sometimes necessary to reduce the volume of the solutions, particularly when the solutions contain considerable amounts of alkali salts. As some handbooks state that tellurous acid can be absorbed by ferric hydroxide and, therefore, can be coprecipitated with this substance (9, p. 170), some experiments of this kind were performed. The precipitation of tellurium from the ferric chloride solutions, however, was not complete; 1 mg. of tellurium in 10 ml. of final volume was very incompletely precipitated, about 0.5 mg. remaining in solution in spite of excess of the reducing agents.

The influence of sulfate ions on the precipitation of tellurium has been recently discussed by Goto and Ogawa (5), who found that the results obtained were too low when 5 ml. of sulfuric acid were present in 100 ml. of solution. As very dilute tellurium solutions must be concentrated before precipitation of tellurium and most of the sulfate ions must be removed, the ferric chloride was replaced by aluminum chloride. The results (Table I) show a very good accuracy, with complete precipitation of 0.2 mg. of tellurium by ammonia from a solution containing 10 mg. of aluminum hydroxide expressed as alumina. About 20 parts of aluminum hydroxide expressed as alumina are necessary to coprecipitate one part of tellurous acid even in the presence of large amounts of sulfate ions. If tellurium is to be precipitated from a solution containing sulfuric acid in large amounts, the simplest procedure is to neutralize the solution with alkali hydroxide and then to add hydrochloric acid and the reducing agents in the prescribed amounts. The alkali sulfate, when formed in large amounts, however, causes some trouble, and it should be removed, preferably before the reduction, by an enrichment with aluminum hydroxide.

Table I. Concentration of Tellurium by Coprecipitation with Aluminum Hydroxide

(Varying ratios of alumina to tellurium and volumes of precipitation. Starting volume 200 ml.)

Sulfuric Ions, Grams	Vol. at Precipitation, Ml.	Alumina, Mg.	Te Taken, Mg.	Te Found, Mg.	Difference, Mg.
..	25	15	1.00	0.94	−0.06
15	25	15	1.00	0.96	−0.04
..	15	20	1.00	0.99	−0.01
15	15	20	1.00	0.91	−0.09
..	10	10	1.00	0.94	−0.06
15	10	10	1.00	0.62	−0.38

Table II. Spectroanalytical Control of Determinations

(Tellurium coprecipitated with aluminum hydroxide from 100 ml. of solution)

	Te Present, Mg.	Te Found, Mg. Spectroanal.	Te Found, Mg. Chem.	
Control solution	1.00	1.0	0.97	
	0.50	0.50	0.52	
	0.20	0.20	0.18	
In 100-g. sulfur sample				
I		3.2	3.6	Ordovician sulfur
II		<0.1	<0.1	Volcanic sulfur

DETERMINATION OF TELLURIUM IN SULFUR

The methods described above enable the determination of very small amounts of tellurium in sulfur.

The sulfur was burned in the glass apparatus shown in Figure 1.

A quartz boat containing the weighed sample was placed in a tube consisting of a wider and a narrow part; the narrow end was connected with a double bubble counter. This tube was placed inside an outer tube, and connected so that air or oxygen currents could pass outside the inner tube as well as within it over the quartz boat containing the sulfur. The outer tube was connected with absorption vessels by glass joints. The sulfur sample was ignited immediately before the apparatus was connected, and the two currents were regulated, the oxygen in the inner tube and the air in the external one, so that the sulfur burned quietly without sublimation. The absorption vessels for the gas leaving the reaction tube contained potassium hydroxide solution (10%).

When combustion was complete, the absorption vessels were emptied and washed with a small volume of dilute nitric acid and the combined solutions were evaporated to dryness repeatedly with added hydrochloric acid. Some insoluble substance, chiefly silicic acid originating from dust occurring in the sulfur, was removed by filtration, a sufficient amount of aluminum chloride was added, and the aluminum hydroxide was precipitated, filtered off, and dissolved in hydrochloric acid. The whole amount of tellurium present was now concentrated in this aluminum chloride solution. Some other metals could be coprecipitated with the aluminum hydroxide, but only selenium necessitates further separation operations. After precipitation of tellurium by reduction as described above, the coprecipitated selenium was determined according to the method of Keller (8). In the majority of cases, it is necessary to dissolve the weighed tellurium in nitric acid, remove this acid by evaporation with hydrochloric acid, and repeat the tellurium precipitation.

In order to make sure of the complete coprecipitation of the tellurium, some of the determinations were controlled spectroanalytically (Table II). The values found gravimetrically and spectroanalytically show good accuracy, for synthetic solutions as well as sulfur samples.

ACKNOWLEDGMENT

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Procedure for Routine Assay of Tritium in Water

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A precise method for the assay of the tritium content of tritiated water has been developed. The method involves reduction of ca. 1 millimole of the tritiated water to tritiated hydrogen which is transferred to an ionization chamber. The ion current activity of the tritium is measured with a vibrating reed electrometer. Analyses are reproducible to ±2% over a thousand-fold activity range, from 10^{-6} to 10^{-9} curies per millimole.

IN VIEW of the increasing importance of tritium in chemical and biological studies (*5, 7*) a precise method is reported for the routine assay of tritium in water samples. The method involves reduction of approximately 1 millimole of water to hydrogen with amalgamated magnesium at 400° C. in a borosilicate glass bomb. The hydrogen produced is transferred to an ionization chamber in which the tritium activity is determined with a vibrating reed electrometer.

Because excellent papers have appeared recently on reduction of organic compounds to gas for tritium assay (*8*) and on ion current measurement (*1, 9*) this procedure is limited to reducing water and to transferring the gas to an ionization chamber. This is a simplification of the procedure described by Henriques and Margnetti (*4*).

EXPERIMENTAL

Equipment and Materials. Aliquots of a stock solution of tritiated water of activity 77 μc. per ml. were quantitatively diluted by weight in order to prepare a series of water solutions containing a range of tritium activities. The magnesium was Baker and Adamson C.P. granular grade. The tank hydrogen was Airco regular grade of 99.5% purity.

A vacuum line of standard design, employing a one-stage oil diffusion pump backed by a Welch Duoseal oil pump provided a vacuum of 10^{-4} to 10^{-5} mm. of mercury. The line designed for the transfer of tritiated hydrogen to the ionization chamber is shown in Figure 1. The Borkowski type ionization chambers (*1*) and the vibrating reed electrometer (Model 30) coupled to a Brown potentiometric strip-chart recorder with multiple range recording (Model 39) are of standard design available from Applied Physics Corp., Pasadena, Calif., for carbon-14 analysis (*6*). All equipment was in an air-conditioned room maintained at 26° ± 1° C. and < 45% humidity.

Reduction of Tritiated Water. A sample of tritiated water (13 to 18 mg., 0.7 to 1.0 mmole) was sealed in a small weighed borosilicate glass ampoule constructed with a break tip (Figure 2). The filling was accomplished by placing the open tip of a heated ampoule in the tritiated water and allowing a sample of the water to be drawn into the ampoule as it was allowed to cool. The tip was sealed in a small flame. The weight of the sample was determined using an ordinary analytical balance and the ampoule was placed in the bomb shown in Figure 2, with 0.3 gram of granular magnesium and 0.3 gram of mercury. The bomb was constructed of 10-mm. tubing (ordinary Pyrex, Corning No. 774) with one end sealed with a break tip. The bomb was connected to the auxiliary vacuum line with pressure tubing, evacuated to a pressure of 0.05 mm. of mercury, sealed at the constriction, and shaken to mix the mercury and the magnesium thoroughly and to break the ampoule containing the tritiated water. The bomb was then placed in a muffle furnace (Blue M electric furnace, Model M225A) regulated at a temperature of 400° to 410° C. for 1 to 2 hours.

Transfer of Tritiated Hydrogen to Ionization Chamber. The vacuum line is shown in Figure 1. The bomb holder, *A*, is constructed with a 19/38 standard-taper ground-glass joint, *a*. Handle *B*, designed to fit into a side arm on the bomb holder and constructed with a ring tip, was made from an inner 10/30 standard-taper ground-glass joint with sealed tube. Toepler pump, *T*, consists of bulbs that may be filled with mercury from a reservoir so that the volume of the line may be varied. The total volume of the line-up to the stopcocks, *F* and *G*, and calibration mark *b*, including manometer *M*, is approximately 60 ml., while the volumes between *b* and *c*, *c* and *d*, and *d* and *e* are 50, 50, and 100 ml., respectively. These volumes were determined precisely by application of the gas law. A measured volume of dry air at a known pressure was introduced into the evacuated system from gas buret, *K*, and the pressure in the system was measured on manometer *M*. This manometer is 1 meter high and the quantity of mercury in it was adjusted for each reading, so that the right arm of the manometer was always set at the same point. The total volume between stopcocks *G* and *H*, and in the ionization chamber, *C* (approximately 250 ml.), was also determined precisely by the calibration procedure.

The bomb, after cooling and weighing, was placed in bomb holder *A*, so that the tip of the bomb fitted into the hole in the ring tip of handle *B*. The remainder of the bomb was surrounded with copper gauze to prevent shattering of the bomb holder when the bomb was broken. The system including the ionization chamber was evacuated to a pressure of at least 10^{-2} mm. of mercury, and all stopcocks were then closed. By twisting handle *B*, the bomb tip was broken. Stopcocks *D* and *E* were opened and with the mercury level in Toepler pump *T* adjusted to calibration mark *b*, the pressure was determined on the manometer. The number of moles of hydrogen gas was calculated from the gas law using the room temperature, the measured pressure and known volume (corrected for the volume displaced by the bomb which was calculated from the weight of the bomb and the density of 2.24 grams per cc. for glass). As a check, the mercury level in Toepler pump *T* was adjusted at other calibration points (*c*, *d*, or *e*), so that the amount of hydrogen could be calculated using pressure readings at other calibrated volumes.

The tritiated hydrogen was now expanded into the ionization chamber, *C*, by opening stopcocks *G* and *H*. In order to eliminate the fractionation of tritium by diffusion in the line, the mercury in Toepler pump *T* was moved up and down several times between levels *b* and *e*, and the gas subsequently allowed to stand for 15 minutes to permit equilibration of the tritium and hydrogen before commencing the next operation. The fraction of the tritiated hydrogen sample introduced into the chamber can be regulated somewhat by adjustment of the mercury level in the Toepler pump, *T*, to different calibration levels.

The ionization chamber, *C*, was filled to atmospheric pressure with tank hydrogen by use of mercury valve *V*. The details of construction and operation of this valve are described by Neville (*6*). The valve from the hydrogen tank was opened (the rate

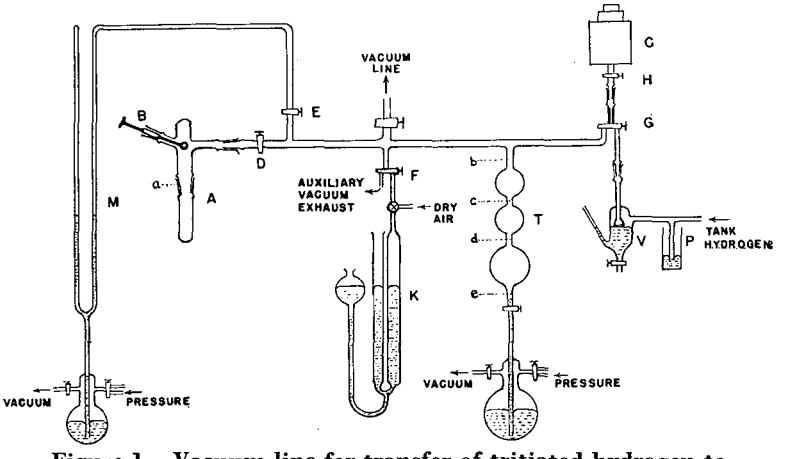

Figure 1. Vacuum line for transfer of tritiated hydrogen to ionization chamber

of flow of hydrogen had been previously adjusted so that C filled to atmospheric pressure in 10 to 15 seconds) and stopcock G was turned so that the tank hydrogen was allowed to flow into C through the medium-porosity fritted-glass disk of valve V. As soon as the pressure in C reached atmospheric, as indicated by cessation in the movement of the mercury in valve V and by the recommencement of bubbling of hydrogen through safety bubbler P, stopcock H was closed, and the chamber, C, was removed. Although counting characteristics vary considerably with the internal pressure with certain gases in the chamber (*1*), this effect is reported as unimportant for hydrogen in the region of atmospheric pressure (*9*).

Tritium Assay. The ionization chamber was connected to the vibrating reed electrometer and the activity determined in the standard manner (*1*, *9*) by measurement of the rate of charge of a condenser or of the voltage drop across a standard high precision resistance, using 180-volt ion collecting potential across the chamber. To calculate the specific activity, the measured activity was divided by the millimoles of tritiated hydrogen. The tritiated hydrogen was calculated from the sample weight (in millimoles of hydrogen) times the volume fraction of the ionization chamber compared to the volume of the whole system. Volumetric measurement of the millimoles of hydrogen gas is employed only as a check on the yield in reduction and consequently measurement of the gas pressure is not necessary for routine analysis.

On completion of the assay, the tritiated hydrogen gas was evacuated from the chamber and the chamber was flushed at least three times by filling with dry air and re-evacuating. The vacuum line was also flushed in a similar manner, employing an auxiliary vacuum system for exhausting the tritiated hydrogen. All tritiated hydrogen gas was exhausted into a ventilating system and the bomb with a small amount of adsorbed tritium was discarded (*8*). No contamination of the vacuum line or the ionization chambers with tritium was detected.

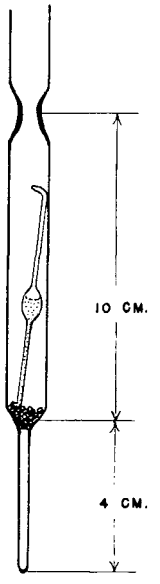

Figure 2. Construction of bomb

RESULTS

A study of the effect of time of heating on the yield of gas and on the specific counting rate was made (Table I). The yield of gas appeared essentially quantitative after 5 minutes at 400° C., and was almost invariably greater than 100% after 1 to 2 hours of heating. A considerable amount of gas (of the order of 5 to 10% of the total amount obtained from 18-mg. water samples) was found to be evolved when only magnesium amalgam was heated in an evacuated bomb. Although this suggests that the reduction of water samples is not over 90 to 95% complete, the effect on reproducibility seems negligible, since the specific counting rate obtained after 1 to 2 hours of heating is reproducible to 1 to 2% (Tables I and II). That some tritium is retained in the reduction mixture even after 2 hours of heating was determined from the fact that reduction of samples of the same tritiated water using zinc and nickelic oxide at 640° C. (*8*) yielded gas of a specific counting rate about 12% higher. Although the reduction appeared to have reached its maximum value after only a few minutes, the highest yield of tritium in the gas was obtained only after approximately 1-hour heating. The reduction mixture should not be heated beyond 2 to 3 hours, since loss of gas occurs (*5*).

Table I. Effect of Time of Heating at 400° C. on Yield of Gas and Specific Counting Rate

Heating Time, Min.	Water Sample Size, Mg.	Gas Yield[a] %	Specific Counting Rate, Mv. per Mmole: Based on mmoles of gas	Specific Counting Rate, Mv. per Mmole: Based on wt. of water
5[b]	21.1	47.2	263	125
5[b]	16.7	51.4	263	135
5	14.3	106.6	462	493
5	15.2	104.1	492	513
15	15.0	106.2	487	517
16	15.8	102.7	499	512
15	16.8	101.3	502	509
15	16.8	104.6	487	510
25	16.7	98.4	506	498
45	21.6	96.2	535	514
47	14.8	102.8	514	533
60	13.9	101.5	511	520
120	14.1	103.7	512	530
120	14.5	100.5	515	519
1020	15.1	87.9	528	463
1200	21.0	88.5	512	453

[a] Based on weight of water sample.
[b] At 180° C.

The usefulness of the tritium assay procedure was tested by analyzing a series of water samples having an activity variation of a thousandfold. The mean specific counting rate and average deviation from the mean for each solution are reported in Table II. The method employing voltage drop across a standard resistance was more precise at high activity levels. In general, a precision of better than ±2% was obtained for solutions of activity of over 10^{-2} μc. per mmole. A slightly lower precision was obtained at the 10^{-3} μc. per mmole level. The specific counting rate was found to be a linear function of the tritium concentration (*9*) with a precision of the order of ±1 to 2%.

In general, the reproducibility of the specific counting rates expressed in terms of millimoles of water was better than that expressed in terms of millimoles of gas. This is reasonable in view of the fact that the large amounts of gas evolved from the magnesium should make the number of millimoles of gas more variable than would be expected from an error in weighing the water. In routine analysis, measurements of the millimoles of gas should not be necessary and the results may be reported solely in terms of millimoles of water.

In any given tracer experiment the highest possible precision in measuring the specific counting rates by this procedure will

Table II. Tritium Assay Results[a]

Tritium Activity in Water, μc. per Mmole	Specific Counting Rates: Mv. per Mmole[b] Gas[d]	Mv. per Mmole[b] Water[e]	Mv. per μc. Gas[d]	Mv. per μc. Water[e]	Mv. per Sec. per Mmole[c] Gas[d]	Mv. per Sec. per Mmole[c] Water[e]	Mv. per Sec. per μc. Gas[d]	Mv. per Sec. per μc. Water[e]	No. of Samples
1.39	518 ± 6[f]	524 ± 9	372 ± 4	377 ± 6	29.3 ± 0.4	29.8 ± 0.5	21.1 ± 0.3	21.4 ± 0.3	8
0.276	101 ± 2	103 ± 1	368 ± 5	375 ± 2	5.61 ± 0.09	5.72 ± 0.02	20.4 ± 0.3	20.7 ± 0.1	5
0.0691	26.5 ± 0.4	26.3 ± 0.4	384 ± 6	381 ± 5	1.49 ± 0.04	1.50 ± 0.02	21.6 ± 0.3	21.7 ± 0.3	4
0.0155	[g]	[g]	[g]	[g]	0.317 ± 0.002	0 327 ± 0.001	20.4 ± 0.1	21.1 ± 0.1	3
0.00144	[g]	[g]	[g]	[g]	0.0303 ± 0.0008	0.0314 ± 0.0008	21.0 ± 0.6	21.8 ± 0.5	4
	Means		375 ± 6	378 ± 2			20.9 ± 0.4	21.3 ± 0.4	

[a] Data obtained by heating water samples of 16- to 20-mg. weight at approximately 400° C. for times varying from 2 to 3 hours. Two ion chambers were used interchangeably. Data were obtained by two different workers (V. P. K. and W. A. S.).
[b] From voltage drop across standard 10^{12}-ohm resistance.
[c] From rate of charge of a condenser.
[d] Based on mmoles of gas.
[e] Based on mmoles of water sample.
[f] Precision expressed as average deviation from mean.
[g] Ion current too low to use voltage drop method.

probably be obtained by carrying out the reductions for 1 to 2 hours at 400° C. In addition, the size of the water samples should be in the range of 13 to 18 mg. and should not be over 20 mg. In a few cases in which large samples of water were used (in the order of 25 to 30 mg.), the yield of gas was only 90 to 95% and the specific counting rate was inconsistent with that obtained from smaller samples.

The lowest level of tritium activity at which measurements were made was 10^{-3} μc. per mmole of water. At this level the ion current produced by the tritium is approximately ten times that produced by the background. With greater care and extended counting periods, activities as low as 10^{-4} μc. per mmole can be measured, although at such a level the precision is expected to be relatively poor because the ion current produced by the tritium is of the same order as the background. The precision at this low level can probably be improved by modifying the apparatus, so that the hydrogen from larger samples of tritiated water can be introduced into the ion chamber (*4*). Tritiated water samples of much higher specific activity than those used in this work also may be analyzed in this apparatus (*9*).

The complete working time for an analysis including weighing the water sample is approximately 45 minutes. The method has the advantage that the only important contamination is in the bomb tube (*8*) which is discarded, so that special procedures such as preconditioning of apparatus (*3*) are avoided. Another advantage is that the sealed tubes are not constructed of special borosilicate glass and do not require the higher temperature heating (*8*). Reduction with zinc at 400° C. (*2*) should also be practical for tritium assay, but it was not examined in this investigation.

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Determination of Tannins and Related Polyphenols in Foods

Comparison of Loewenthal and Pro Methods

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The volumetric permanganate titration and the colorimetric phosphomolybdic-tungstate reduction procedure were compared for pure polyphenols, commercial tannins, and partially purified fruit tannins. The permanganate titration gave significantly higher results for catechol, hydroquinone, pyrogallol, and chlorogenic acid, and lower results for phenol, resorcinol, catechin, and quercetin. With commercial tannin preparations the results were lower but were essentially similar for fruit tannins. Changes in absorption spectra during titration with permanganate and changes in redox potential are reported.

THE available methods of analysis for tannins have been critically reviewed by Joslyn (*5*), Mitchell (*7*), and Nierenstein (*9*). For the determination of tannins and related polyphenols present in fruits and fruit products, the two most widely used are the Loewenthal volumetric procedure (*1*) and the Folin-Denis colorimetric procedure (*10, 12*). In the present investigation these two general methods of determination of tannins were compared for a series of known compounds as well as a group of commercial tannins and isolated fruit tannins.

The volumetric permanganate method developed by Loewenthal (*6*), after some modification, was adopted as an official method for tannins in coffee and tea, spices and condiments, and wines. Loewenthal improved the method originally proposed by Monier (*8*), in which the tannin was titrated directly with potassium permanganate, by carrying out the titration in the presence of indigo carmine. Loewenthal stated that the indigo carmine acted not as an indicator, but as a regulator, because in its presence only those compounds were oxidized whose rates of reaction with permanganate were faster than with indigo carmine. To determine the nontannin equivalent, a gelatin solution with salt was used to precipitate the tannins. Proctor (*11*) repeated Loewenthal's work and made improvements in standardization of the permanganate solution.

In the determination of tannin in coffee and tea, gelatin is used to separate tannin from solution and other permanganate reducing substances present. In wines, purified bone black is used to absorb tannins and coloring matter. During the titration the permanganate is added in 1-ml. quantities with constant stirring until a green color is reached. The titration is then continued dropwise to a golden yellow end point. The inclusion of coloring matter with tannin in the analysis of wine was supported by Bate-Smith and Swain's (*3*) contention that leucoanthocyanins are closely related chemically to the catechins and should be regarded as prototypes of condensed tannins. Williams (*14*) showed by paper chromatography that leucoanthocyanins, catechins, and chlorogenic acid, present in cider, all contribute to the total tannin titration. Hide powder removed the major part of the simple polyphenols present and the bulk of the other more complex phenolic materials. Barua and Roberts (*2*) criticized the Loewenthal procedure because of the errors involved in the arbitrary end point. They found the method became completely unreliable with oxidized tannins, because the titration was carried to a different end point owing to decreases in the rate of oxidation.

The Folin and Denis (*4*) colorimetric procedure as modified by Rosenblatt and Peluso (*12*) and Pro (*10*) is official for tannins in distilled liquors. The blue color which is produced only after addition of alkali to a mixture of the Folin-Denis reagent with

tannins was assumed by Folin and Denis to be formed by reduction of the phosphomolybditungstic acid and the change in color of the reduction product of the latter on addition of alkali. Aliphatic compounds did not give the reaction, but the reaction was positive with all compounds having an oxyphenyl bond, and was believed to be specific for phenols. Subsequently it was shown that ascorbic acid will also react with the Folin-Denis reagent and in tannin extracts containing ascorbic acid high results for tannin will occur (*13*).

EXPERIMENTAL METHODS

The fruits used in the investigation were stored for periods of a few months to a year at −18.5° C. before use.

Preparation of Crude Fruit Tannins. The fruit tannins were prepared by blending 50 grams of fruit with 250 ml. of 95% ethyl alcohol and refluxing for 15 minutes. After filtration 20 ml. of saturated neutral lead acetate were added, and the solution was adjusted to pH 7 with 10% sodium hydroxide solution and left overnight. The precipitate was then centrifuged out, and washed three times with small amounts of water and the lead removed with Amberlite IR-120 in the hydrogen form. After removal of the resin and filtration through Whatman No. 2 paper, the solution was evaporated to 50 ml. on the steam bath. Fifty milliliters of water were then added and the solution was evaporated again to 50 ml. to remove all ethyl alcohol. The solution was then made up to 100 ml. in a volumetric flask and analyzed. In addition to the determination of tannin, the total solids content in an aliquot of this material was also determined, by first evaporating on a steam bath and then drying at 70° C. in a vacuum oven in the presence of asbestos.

Folin-Denis Colorimetric Determination. Determinations were carried out as described by Pro (*10*) with a Klett Summerson photoelectric colorimeter, standardized against a blank containing all the reagents. The equivalent in terms of U.S.P. tannic acid was read from a calibration curve made in the same way with pure tannic acid.

Loewenthal Titration. The potassium permanganate and indigo carmine solutions, and the purified bone black were prepared as described in the AOAC official methods of analysis (*1*). The bone black after washing was mixed with distilled water to give a final volume of 1 liter.

Determinations were carried out mainly as described by Joslyn (*5*). In the determination a 5- to 25-ml. aliquot of the tannin solution was added to 500 ml. of water in an 800-ml. beaker. Five milliliters of indigo carmine solution were added. The permanganate was then added in 1-ml. portions with vigorous stirring until the solution turned green. The titration was then continued dropwise to a golden-yellow end point. To determine the nontannin reducing materials, twice the amount of the aliquot taken before was mixed with 7 ml. of the bone-black suspension and made to 100 ml. After standing for 15 minutes, the solution was filtered through a Whatman No. 2 filter paper, and the first portion of the filtrate discarded. Fifty milliliters of the clear filtrate was then titrated as before. The difference in the two titers was taken as a measure of the tanninlike materials present in the original aliquot. Aliquots were chosen to give a total titration of 6 to 8 ml. before and 4 to 5 ml. after treatment with charcoal.

Table I. Comparison of Loewenthal and Pro Procedures

	Pro[a]	Loewenthal[a]	Difference[b]
Phenolic Substances			
Hydroquinone	1215	2946	+41.6
Chlorogenic acid[c]	725	950	+13.4
Catechol	2125	2437	+ 6.8
Pyrogallol	1738	1963	+ 6.1
Catechin[d]	1250	1054	− 8.5
Quercetin	1615	1306	−10.6
Phenol	1487	955	−21.8
Resorcinol	1625	489	−53.7
Commercial Tannins			
Tannic acid, U.S.P.	1000	1000	0
Tannic acid, Eastman Kodak	1217	1147	− 3.0
Quebracho wood tannin[e]	833	639	−13.2
Grape seed tannin	900	679	−14.1
Fruit tannins			
Green persimmon	53	57	+ 3.6
Green apple	144	146	+ 0.7
Green peach	155	157	+ 0.6
Ripe apple	214	216	+ 0.5
Ripe persimmon	40	40	0
Ripe peach	69	66	− 2.9
Green pear	94	83	− 6.7
Ripe pear	162	140	− 7.3

[a] Expressed as equivalent weight of U.S.P. tannic acid, mg. per gram dry weight.
[b] Expressed as percentage by which results with Loewenthal procedure were higher (+) or lower (−) than average of results with two methods.
[c] Obtained from Western Regional Research Laboratory, Albany, Calif.
[d] Supplied by E. C. Bate-Smith.
[e] Obtained from Eastern Regional Research Laboratory, Philadelphia, Pa.

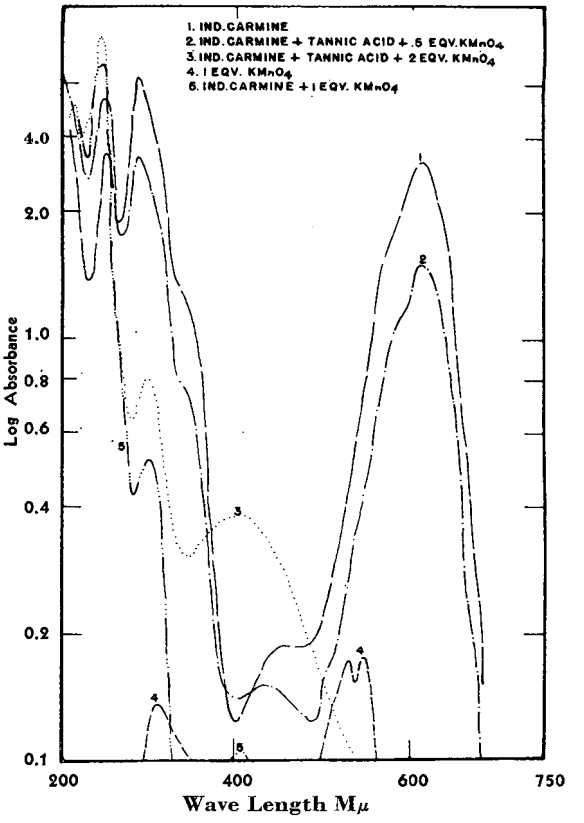

Figure 1. Absorption spectra of indigo carmine, potassium permanganate, and tannin solutions at various levels of oxidation

Absorption Spectra Measurements. For the measurement of absorption spectra a Beckman Model DU spectrophotometer with 1-cm. quartz absorption cells was used. Distilled water was used as a blank.

The solutions were made up exactly as in the actual determination, except that dilutions were made with 400 ml. of water, so that the final solutions could be made up to 500 ml. in a volumetric flask. Whenever indigo carmine was not present 5 ml. of a solution containing 50 ml. of concentrated sulfuric acid per liter were added to the reagents to give a pH of approximately 2. When it was necessary to make dilutions during the absorption spectrum measurements a diluting solution containing 0.50 ml. of concentrated sulfuric acid per liter was used. This solution also served as a blank. The pH of all the solutions ranged between 1.99 and 2.08. The tannic acid solution used was made by dissolving 0.1 gram of U.S.P. tannic acid in 100 ml. of water. Five milliliters of this solution were used in the measurements.

Measurement of Oxidation Reduction Potential Changes in Loewenthal Volumetric Permanganate Procedure. APPARATUS. A pint jar was fitted with a rubber stopper assembly containing two platinum wire electrodes, a sintered-glass bubbling tube, a saturated potassium chloride–agar bridge, a stirrer, and a buret. A saturated potassium chloride calomel electrode was used, and the oxidation-reduction potential readings were made on a Leeds & Northrup No. 7661-A-1 Universal pH potentiometer assembly.

SOLUTIONS. The potassium permanganate, indigo carmine, and tannin solutions used were the same as those described for Loewenthal titration.

PROCEDURE. Five milliliters of indigo carmine were added to 300 ml. of distilled water. With stirring and nitrogen bubbling—to ensure immediate mixing—potassium permanganate was added 1 ml. at a time. After each addition the oxidation-reduction

potential was periodically observed until it leveled off to a constant value and then another aliquot of permanganate was added.

DATA AND DISCUSSION

The results for the series of compounds and materials analyzed are given in Table I. In the table U.S.P. tannic acid was used as reference material and all the results are therefore expressed in terms of the equivalent weight of tannic acid present in 1 gram of material used.

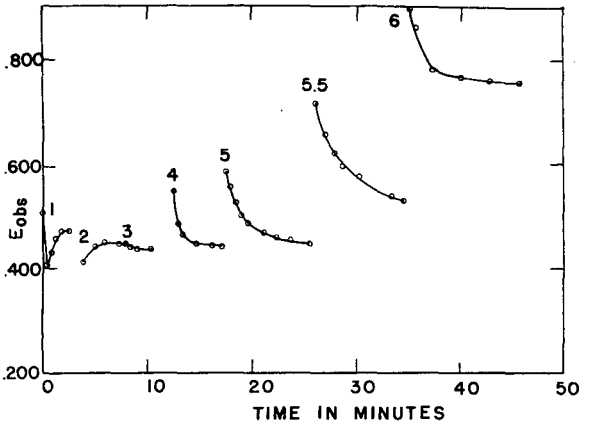

Figure 2. Changes in redox potential of indigo carmine solution during oxidation with potassium permanganate

The Loewenthal procedure gave very high results with hydroquinone, and very low results with resorcinol. This low value obtained with resorcinol could be due to impurities, because Williams (*14*) showed that resorcinol does not react with permanganate during the course of the Loewenthal titration.

Of the pure compounds tested, the smallest differences in the two methods were found with catechol, catechin, quercetin, and pyrogallol. Of these compounds pyrogallol and catechol gave the highest results with the Loewenthal procedure and catechin and quercetin gave the highest results with the Folin-Denis reagent.

With the commercial tannins much smaller differences were found, and with the three tannins tested the colorimetric procedure gave higher results throughout. A better agreement between the two methods was found with the fruit tannins, where the differences ranged between 3.6 and −7.3%.

There was a close agreement between the two methods with the fruit tannins. Although not probable, such an agreement was possible because a mixture of compounds was determined. Individual members of this mixture would obviously vary in ease of oxidation and in the number of hydroxyphenyl groups present. In the mixture results would therefore depend on the types of compounds present, and the ratio of one to the other. This was very well illustrated when the values for the pure compounds were determined. Catechol, for example, gave a value higher by 312 mg. per gram with the Loewenthal procedure, while quercetin gave a result lower by 309 mg. per gram with the colorimetric procedure. In a 1 to 1 mixture these two compounds would agree closely when tested by the two procedures.

The changes in absorption spectrum occurring during the course of the Loewenthal titration were also investigated and these results are plotted in part in Figure 1.

In the visible region the curves for indigo carmine and indigo carmine plus tannic acid were similar and only one of these curves was therefore plotted in Figure 1. From the figure it can be seen how the maximum at approximately 610 mμ disappears during the course of the titration, while the minimum at approximately 400 mμ changes into a maximum as the color of the solution changes from a blue through a green to a golden yellow color.

The behavior of the spectra at 390 to 420 mμ is of special interest in that it seems to have a minimum with the reagents alone—that is, with either indigo carmine or permanganate. The formation of an oxidation product of indigo carmine however changes this minimum into a maximum. The tannic acid which is oxidized before the indigo carmine did not seem to give a maximum at the same position.

The change in oxidation-reduction (E observed) with time and volume of permanganate added is plotted in Figures 2 and 3. The numbers above each line indicate the volume of permanganate added at that point. A direct potentiometric titration of indigo carmine alone or in the presence of tannin could not be made because of the changes in redox potential occurring after the addition of each aliquot of permanganate. These changes following the addition of each successive milliliter of permanganate were measured. In Figures 2 and 3 the numbers above the plot of redox potential against time indicate the total volume of permanganate added.

Figure 2 represents the changes in redox potential during the titration of 5 ml. of indigo carmine in 300 ml. of water in absence of added tannin. No change in color was observed after the addition of the first 3 ml. of permanganate solution, after 4-ml. addition, the solution became light blue, after 5-ml. addition, green, and after 5.5-ml. addition, yellow in color. The observed redox potential at first increased during standing but after 3 ml. of permanganate it decreased with time. The rate of decrease was sharpest at 4 ml. Both the initial and final redox measurements increased with volume of permanganate added but there was no indication of a noticeable change at the end point which could be used as a basis for a potentiometric determination of tannins.

Figure 3 represents the changes in redox potential with time during the titration of 10 ml. of 0.1% U.S.P. tannic acid and 10 ml. of indigo carmine solution added to 300 ml. of water. No detectable change in color of solution occurred after the addition of the first 14 ml. of permanganate solution, after 15 to 16 ml. the solution became light blue, after 17 to 18 ml. it was green, and at 19 ml. it became yellow.

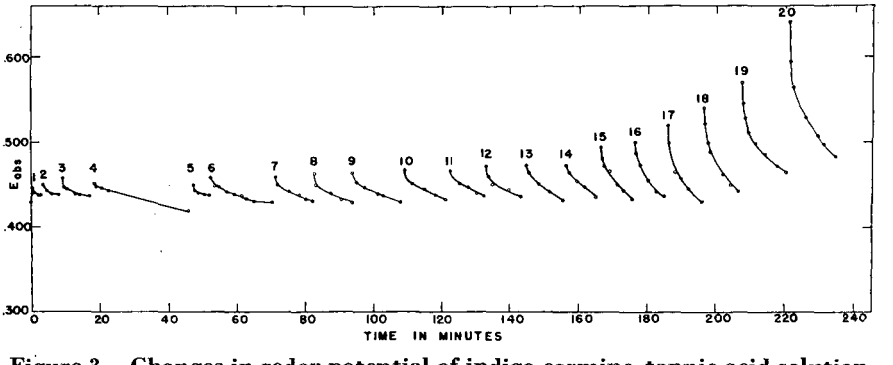

Figure 3. Changes in redox potential of indigo carmine–tannic acid solution during oxidation with potassium permanganate

The initial redox values changed but little with volume of permanganate added up to about 14 ml. and then gradually reversed. The rate of drop in redox potential increased with volume of permanganate added up to 20 ml. (1 ml. over the end point). Permanganate was still being reduced after the end point was reached, indicating incomplete oxidation at this point.

CONCLUSIONS

A comparison of the results obtained brings out the highly empirical nature of the two methods examined. The fairly close agreement with the phenolic substances, isolated from fruit when tannic acid was used as reference material, was due to the mixture of different polyphenolic materials. This agreement varies from one mixture to the other and therefore from one fruit to the other.

Inspection of the absorption spectra during the course of the Loewenthal titration suggests the possibility of developing a colorimetric determination of the end point to replace the present difficult visual determination.

Results of the redox potential measurements show that a potentiometric determination of the end point of the permanganate titration is not feasible.

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Rapid Microprocedure for Determination of Mercury in Biological and Mineral Materials

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A rapid procedure to determine microamounts of mercury in soil and biological materials was needed in an investigation of organic mercurial fungicides. A method is described which requires only standard laboratory equipment, is specific and relatively rapid, and has an accuracy within 1 γ or 5% at the 1- to 100-γ level. Common metallic ions do not interfere. This procedure may be used to determine mercury in small amounts of soil, plant and animal materials, and in air.

A RAPID and specific quantitative microprocedure for mercury in soil and biological materials was needed in an investigation of organic mercurial fungicides. Previous soil analysis for mercury was reported by Stock and Cucuel (*15*), who separated the mercury by pyrolysis and measured the mercury globule formed following electrolytical deposition. The determination of mercury in biological material at the microlevel is usually based on the color reaction between mercuric mercury and diphenylthiocarbazone (dithizone) in chloroform, the colored mercury dithizonate being measured in a photometer. This reaction together with the limitations is adequately discussed by Sandell (*11*) and Snell and Snell (*13*). Klein (*3*, *4*) has made careful studies of this procedure. Snell and Snell pointed out that numerous modifications of this reaction leave much to be desired. The proposed method has comparable accuracy and fewer steps than many of the available procedures.

The sample is prepared by digestion in concentrated sulfuric acid with dropwise additions of 50% hydrogen peroxide. This digestion mixture was used in the determination of mercury by Stettbacher (*14*), Graham (1), and recently by Melles and de Bree (*6*). The analysis of the solution for mercury is based on the same chemical reaction as one of the procedures for diphenylmercury previously reported by the authors (*7*). An excess of an alcoholic solution of a diorganic mercurial reacts with a weak acid solution of mercury to form two molecules of the corresponding organic mercury compound. The resulting organic mercurial is measured by the color formed from reaction with dithizone.

REAGENTS

Sulfuric acid, concentrated and 1.8*N*.

Hydrogen peroxide, 50 and 90%. Precautions for handling this reagent are given by Shanley and Greenspan (*12*). (Both peroxide samples supplied by Buffalo Electrochemical Co.)

Potassium permanganate solution, 3.0%.

Hydroxylamine hydrochloride solution, 20.0%.

Sodium chloride, 1*N*.

Sodium acetate, 4*N*.

Accutint indicator paper, No. 60.

Ditolylmercury solution. Twenty milligrams of ditolylmercury (Eastman Kodak No. 1448) are dissolved in 200 ml. of neutral redistilled ethyl alcohol by heating under reflux on a steam bath. This reagent is stable for at least 6 weeks when stored in a dark bottle. Chilling is avoided to prevent formation of crystals.

Acetic acid, 0.3*N*.

Dithizone solution. Eastman Kodak white label diphenylthiocarbazone is dissolved at the rate of 1 mg. per ml. in chloroform and stored under refrigeration. Dilutions in chloroform are made daily to give the desired working range. Using a 4 to 120 dilution, 0 to 8 γ of mercury may be determined, while a 10 to 120 dilution gives a 17- to 27-γ range.

Standard mercury solution, 1000 γ per ml. Mercuric chloride is dissolved at the rate of 0.1354 gram per 100 ml. of 1*N* sulfuric acid. Measured amounts of a 1 to 100 dilution are used to prepare a standard curve each day.

The sodium chloride, sodium acetate, and hydroxylamine hydrochloride solutions are extracted with dithizone, followed by chloroform. The water and chloroform are redistilled in a glass still.

PREPARATION OF SAMPLE

The sulfuric acid–hydrogen peroxide digestion procedure must be modified for different types of material because very rapid reactions result in loss of mercury. The modifications necessary are determined by the organic matter and moisture content, and by the physical form of the sample. Each analyst should re-

cover known amounts of mercury from the particular type of material involved. One person can conduct several digestions simultaneously.

Soil. A 1-gram sample of soil, that has been passed through a 32-mesh screen to remove rocks, roots, and other extraneous material, is weighed into a 125-ml. standard-taper flask and 5 ml. of concentrated sulfuric acid are added. For soil containing 15% or more organic matter 10 ml. of acid are used. The flask is attached to a West condenser and is swirled until the soil is mixed completely with the acid. Hydrogen peroxide, 50%, is added dropwise through the condenser and the flask swirled vigorously after each drop. The drops are added at a rate which keeps the mixture bubbling gently but never so there is a large accumulation of peroxide. Hydrogen peroxide, 50%, is added until the solution portion becomes white or pale yellow, or until 2 drops at once cause no reaction. Mineral soils clear rapidly, but dropwise additions for 1.5 hours are sufficient for even the very high organic matter soils. The mixture is heated slowly with the flask on an asbestos board using a microburner. The additions of 50% hydrogen peroxide are continued for 20 minutes and the mixture is boiled for approximately 2 minutes after the last addition. Following a brief air cooling, the flask is immersed in a cold water bath and 15 ml. of redistilled water are added. The solution is mixed and cooled again. Potassium permanganate, 3.0%, is added until the permanganate color remains. The condenser is rinsed with water, and the excess permanganate removed by the addition of 6 ml. of 20% hydroxylamine hydrochloride. The condenser is washed down again. After standing for 15 minutes or longer the mixture is filtered using a medium porosity sintered-glass filter and made to 100-ml. Sodium chloride, 1*N*, is used for the transfer.

Plant and Animal Tissue. A sample low in moisture and in a finely ground form is typified by fish meal. Ten milliliters of concentrated sulfuric acid are added to a 1-gram sample and mixed thoroughly before adding the first drop of 50% hydrogen peroxide. The first few drops must be added very slowly with much mixing to prevent a rapid reaction with visible vapor loss. When the initial foaming has subsided the drops may be added fairly rapidly. Additions are made until the solution is pale yellow or white. If the solution becomes cold and the peroxide is not reacting, the additions may be stopped when the solution is still orange. It is heated slowly on a steam bath with occasional swirling and with no additional peroxide until the excess has reacted. Drops are then added until the solution is clear and almost colorless. The flask is removed from the steam bath and heated cautiously with a microburner. Drops of peroxide are added as the solution darkens and heating is continued until the solution remains white after heating for approximately 2 minutes. Potassium permanganate, 3.0%, and 20% hydroxylamine hydrochloride are added as with soil samples. The solution is transferred using 1*N* sodium chloride and made to 100 ml.

The husk of a narcissus bulb is a sample low in moisture and in the form of scales. This type of sample is soaked approximately 10 minutes in 10 ml. of the acid and mixed thoroughly before the first addition of 50% hydrogen peroxide. It is then treated like the fish meal.

An example of a sample low in moisture and in the form of chunks is kernels of wheat. A 1-gram sample is soaked 10 minutes in 5 ml. of concentrated sulfuric acid. Hydrogen peroxide, 50%, is added dropwise with swirling until approximately 15 drops have been added. The solution will still be black at this point. It is placed on a steam bath and the digestion procedure follows that outlined for fish meal after placement on the steam bath.

The meat of a bulb is an example of high moisture content and chunk form. To a sample not exceeding 3 grams of dry matter are added 10 ml. of concentrated sulfuric acid. The mixture is cooled before addition of 50% hydrogen peroxide. It is then treated in the same way as the wheat kernels, except that 90% hydrogen peroxide is used after the first reaction on the steam bath is over. It will not become colorless on the steam bath and the heating with the burner is begun when the sample is light orange. As there is considerable wax present, the solution must be filtered.

DETERMINATION

An aliquot of the digest containing 0 to 8 or 17 to 27 γ is placed in a 250-ml. separatory funnel containing an equal volume of water, or with small aliquots sufficient water to make a total volume of 75 ml. When 10 ml. of concentrated sulfuric acid are used in the digestion, the water must have a volume of twice the aliquot. With fish-meal samples it is necessary to add at this point 3 ml. of 5*N* sodium chloride for every 25 ml. of the sample used. Sodium acetate, 4*N*, is added with swirling to give a pH of 3.0 to 4.0 as determined by Accutint indicator paper. One milliliter of ditolylmercury solution is added and the separatory shaken 3 times. If the sample is in the 17- to 27-γ range, 2 ml. of ditolylmercury solution are used. After standing 1 minute, approximately 9.5 ml. of chloroform are added and the funnel is shaken for 1 minute. When the volume of solution in the first separatory is 150 ml. or greater the extraction with chloroform is done in two steps. The first is with 8.0 ml. of chloroform and the second with 3.0 ml. The chloroform phase is transferred to a second separatory funnel which contains 25 ml. of 0.3*N* acetic acid. One milliliter of diluted dithizone solution, corresponding to the expected mercury content, is measured into the funnel and shaken for 30 seconds. The chloroform phase is transferred and made to volume in a 10-ml. volumetric tube. The percentage of transmittance is determined with a 620-mμ filter in an Evelyn photoelectric colorimeter. As in the determination of organic mercury compounds by the dithizone procedure (*8*), the unreacted dithizone color is measured.

The amount of mercury present is determined by comparing with a curve prepared by using the standard mercury solution in the above procedure. For the standards the first separatory funnel contains 50 ml. of water, 12.5 ml. of 1.8*N* sulfuric acid, and 3 ml. of 20% hydroxylamine hydrochloride. The standard curve follows Beer's law in the indicated range.

DISCUSSION

In the usual procedures for the determination of mercury in biological material, the digestion is accomplished by a mixture of nitric and sulfuric acids. In the described procedure nitric acid could not be used in the digestion. Some types of samples, notably bulbs, on digestion with nitric and sulfuric acid in the apparatus described by Klein (*4*) form a substance which prevents the reaction between mercury and the diorganic mercurial. This substance could be removed by aeration or steam distillation from acid solution and collected in alkali, but it was not characterized further. High results were occasionally obtained with nitric acid digests which were unexplained.

Using the small samples described in this procedure, the complicated apparatus of Klein (*4*) offered no advantage over the simple flask and condenser. Fuming sulfuric acid was tried in place of the concentrated acid in the digestions. It seemed to offer no advantages, and had the disadvantage of causing a more violent reaction with the peroxide. Much of the success of the digestion is believed to be due to a gentle oxidation, avoiding sudden vigorous reaction when mercury is lost, presumably by expulsion from the condenser.

If soil was digested and filtered without the addition of the chloride ion, the results were always low. With the addition of the chloride it is believed that the complex ion between mercury and chloride forms (*9*). This prevents adsorption of the mercury by the silica or other insoluble material. A large excess of chloride is necessary for the reaction. Fish meal requires an unusually large excess; otherwise the results are low. Three milliliters of 5*N* sodium chloride per 25 ml. of solution give accurate readings and a greater excess gives no higher results.

Mercury has been reported to be adsorbed onto the walls of containing vessels, especially from very dilute solution (*11*). To test the effect of acidity and chloride on the stability of mercury solutions in glass, known amounts of mercury were placed in flasks in solutions of various acidity and chloride concentration. Without chloride there was no appreciable loss of mercury with acidification below 0.3*N*, but as acidity increased the losses became higher reaching 18% at 1.8*N*. Above 1.8*N* the adsorption decreased until with 9*N* acid there was no loss. The mercury was not adsorbed from 0.001 to 9*N* sulfuric acid in a 24-hour period when the solution was 0.3*N* in chloride. The concentration of mercury was approximately 1 γ per ml. of solution.

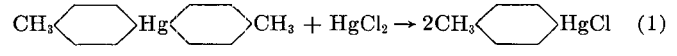
Four dimercurials were found to react quantitatively in the general procedure, but required different reaction conditions. These were di-*p*-tolylmercury, diphenylmercury, dinitrophenylmercury, and bis-*m*-(α,α,α-trifluorotolyl)mercury. The latter two were prepared by known reactions (*2, 5, 10, 16*). The reaction of

diphenylmercury with mercuric chloride required a pH of 4.3. Even at this acidity the procedure had to be carried out rapidly or there was some breakdown of the diphenylmercury. The di(nitrophenyl)mercury required 2 to 4*N* sulfuric acid and the virtual absence of chloride for the reaction to take place. Acidity down to pH of 2.0 could be used with the bis-*m*-(α,α,α-trifluorotolyl)mercury but it had the disadvantage of reacting slowly. The ditolyl compound was chosen for this procedure because it was more stable toward acid and had a more favorable solubility ratio than the diphenylmercury, and the reaction took place more rapidly than with the fluorinated compound. It was also available commercially. Blanks showed no detectable breakdown of the ditolylmercury under the conditions of the procedure.

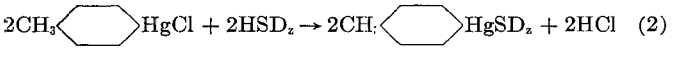
Ditolylmercury is soluble only with difficulty in ethyl alcohol. Other solvents that were tested in the preparation of the ditolylmercury reagent were methanol, isopropanol, acetone, and ethoxyethanol. The first two solvents appeared to be satisfactory but offered no advantages over ethyl alcohol. The latter two solvents, while dissolving the ditolylmercury much more readily, seriously interfered in the determination by causing decomposition of the ditolylmercury.

The spectrophotometric curve for the dithizonate resulting in this procedure has a minimum at 480 mμ, which agrees with a standard *p*-tolylmercuric dithizonate curve and is 10 mμ shorter than the minimum for mercury.

Equations 1 and 2 show that the sensitivity of the new procedure is the same as that of the usual dithizone reaction indicated by Equation 3.



(1)



(2)

$$HgCl_2 + 2HSD_z \rightarrow (D_zS)_2Hg + 2HCl \quad (3)$$

Standard curves prepared by the reactions outlined in Equations 1 and 2 and Equation 3 are identical.

The extraction of the *p*-tolyl mercuric chloride from the reagent mixture to the chloroform is accomplished by one separation, since the solubility is so great in chloroform (Table I). The two extractions with chloroform for the large volume samples are necessary because of the loss of chloroform in the larger amount of water and not because of solubility of the *p*-tolyl mercuric chloride.

Table I. Solubilities of *p*-Tolyl Mercuric Chloride at Room Temperature

Solvent	$CH_3C_6H_4HgCl$, γ per Ml.
Chloroform	13,650
Water	12
Reagent mixture	4

$$\text{Solubility ratio } \frac{CHCl_3}{\text{reagent mixture}} = 3413$$

One thousand micrograms of iron, cobalt, nickel, zinc, cadmium, lead, iron, copper, manganese, and bismuth do not interfere in the determination. Silver requires an additional shake-out of the chloroform phase with a 1*N* sodium chloride solution to prevent mechanical carry-over of the silver chloride precipitate to the dithizone solution.

This analytical procedure can be used to determine mercury in soil, air, and material high in organic matter content, with equally satisfactory results (Table II). The biological materials include both plant and animal matter and represent samples coarse and finely ground and high and low in moisture. Mercury standard was recovered from a reagent mixture that is used in the absorption of mercury from air (*11*).

Table II. Recovery of Added Mercury from Soil or Biological Material

(50 to 100 γ of Hg)

Material	Moisture-Free Org. Matter, %	No. of Samples	Max. Dev., %		Av. Std. Recovery, %
Soil					
Lauren sandy loam	4.9	2		+3.6	101.8
Nisqually gravelly loam	5.5	6	−4.4		96.9
Puyallup sandy loam	6.1	3	−2.4		98.1
Lynden sandy loam	7.2	2		+3.0	101.5
Conroy sandy loam	8.3	2	−1.0		99.0
Salkum loam	11.3	3	−4.1		97.4
Chehalis silt loam	14.8	2	−2.0		98.8
Buckley loam	15.6	3	−3.0		97.1
Cinebar loam	17.4	2	−3.3		97.5
Peat	41.1	5	−2.8		97.9
Fish meal	88.9	17	−3.2	+3.0	98.7
Bulb husks	95.2	6	−2.0		98.8
Bulb meat	98.0	8	−3.5	+2.8	100.1
Wheat (kernels)	98.2	5	−3.4		97.9
Air analysis solution	...	2		+1.3	101.1

Using the Beckman DU spectrophotometer and a 2 to 120 dilution of the dithizone reagent, amounts down to 0.5 γ can be readily determined. With the Evelyn photoelectric colorimeter and using either the 4 to 120 or 10 to 120 dilutions of the dithizone, 1-gram samples of soil containing 0 to 100 γ of mercury were analyzed (Table III). Standard added in the form of organic mercury compounds gave equally satisfactory results. Great care must be used when analyzing samples containing methyl mercury compounds due to the volatility of these materials. The accuracy of the procedure is 1 γ or 5%, whichever is the larger.

Table III. Range of Procedure

(Hg added to soil)

Added As	Added Hg, γ	Found Av., γ Hg	No. of Samples	Maximum Deviation, γ of Hg	
$HgCl_2$	0	0.2	6		+0.7
	5	5.3	6		+1.0
	30	29.9	5	−1.5	+1.6
	50	50.2	3	−0.5	+1.5
	100	96.4	2	−3.8	
ϕHgAc	24	23.5	2	−0.6	
EtHgCl	75.7	73.8	2	−2.1	
MeHgCl	98.3	95.4	3	−3.1	

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Precipitation of Cuprous Hydroxide by Ferrous Ethylenediamine Tetraacetate

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Application of the ferrous ethylenediamine tetraacetate complex as a reducing agent has been extended to the precipitation of copper as cuprous hydroxide. The procedures for the qualitative test and for the turbidity measurement are described. Only silver, gold, iridium, and palladium interfere.

BOTH ferric and ferrous iron form complex ions with ethylene diamine tetraacetate (EDTA), but the ferric complex is much more stable than that of ferrous. The former has a stability constant of log K 25.1 and the latter log K 14.2. In the presence of oxidizing agents such as silver(I), copper(II), etc., the equilibrium

$$FeY^{--} \rightleftharpoons FeY^{-} + e$$

is wholly displaced in favor of the ferric complex. The reducing power of the ferrous complex is stronger than the uncomplexed ferrous ion. Přibil and others (*3*) and Cheng (*1*) have reported that silver ion is reduced to metallic silver by ferrous ethylenediamine tetraacetate complex.

Ordinarily, copper(II) is not reduced by iron(II). However, copper(II) was found to be reduced rapidly by the ferrous sulfate in the presence of ethylenediamine tetraacetate, tartrate, and sodium hydroxide. Silver(I), gold(III), iridium(III), and palladium(II) were also reduced by the ferrous complex to the metals. No other interference was found in the reduction of copper with ferrous ethylenediamine tetraacetate. This new reaction may find application in analysis.

REACTION

The formal potential of the ferric-ferrous system is reported to be the order of −0.77. However, the formal potentials of the ferric-ferrous ethylenediamine tetraacetate system were found to be the order of −0.10 volt (slightly acid medium) and the order of +1.6 volts (in the presence of tartrate and sodium hydroxide).

The ferrous ethylenediamine tetraacetate complex is gradually oxidized by air to ferric ethylenediamine tetraacetate complex, but it changes to ferric complex rapidly in the presence of copper(II)-ethylenediamine tetraacetate complex which is reduced by the ferrous complex. The copper(I)-ethylenediamine tetraacetate complex is not stable. Therefore, copper(I) hydroxide is precipitated in the presence of sodium hydroxide. No copper(I) hydroxide was formed when ammonium hydroxide was used instead of sodium hydroxide. In the reduction of copper(II) with ferrous ethylenediamine tetraacetate complex, the reactions may be written as follows:

$$Cu^{++} + H_2Y^{--} = CuY^{--} + 2H^{+}$$
$$Fe^{++} + H_2Y^{--} = FeY^{--} + 2H^{+}$$
$$FeY^{--} + CuY^{--} = FeY^{-} + CuY^{---}$$
$$CuY^{---} = Cu^{+} + Y^{----}$$
$$Cu^{+} + OH^{-} = CuOH$$

EXPERIMENTAL

Reagents and Instrument. The complexing mixture was prepared by dissolving 10 grams of disodium salt of ethylenediamine tetraacetic acid, 10 grams of tartaric acid, and 10 grams of sodium hydroxide in 100 ml. of water. The ferrous sulfate solution was prepared by dissolving 5 grams of ferrous sulfate septihydrate in 100 ml. of 0.1*N* sulfuric acid. The Fisher Nefluoro-Photometer was used for measuring the turbidity.

QUALITATIVE TEST

One or two drops of the solution to be tested were mixed with one or two drops of the complexing mixture on a white porcelain dish, then one drop of the ferrous sulfate solution was added. A yellowish coloration or precipitate was formed if more than 1 γ of copper were present.

No visible precipitate was observed from 1 or 2 drops of solution which contained 1,000 p.p.m. of any of the following ions: aluminum, ammonium, antimony(III), arsenic(III), barium, beryllium, bismuth(III), calcium, cadmium, cerium(III), cesium, chromium(III), cobalt, columbium, gadolinium, gallium, germanium, hafnium, indium, iron(III), lanthanum, lead, lithium, magnesium, manganese(II), mercury(II), neodymium, nickel, osmium(VIII), potassium, praseodymium, rhodium, rubidium, ruthenium, samarium, scandium, selenium(IV), sodium, strontium, tantalum, tellurium(IV), thallium(I), thorium, tin(III), titanium(III), uranyl, yttrium, zinc, zirconium(III), bromide, chloride, acetate, borate, fluoride, iodide, nitrate, sulfate, molybdate, tungstate, phosphate, and vanadate.

Platinum(IV) gave a grayish coloration. Silver(I), gold(III), palladium(II), and iridium(III) gave a dark precipitate. Gadolinium and samarium showed an intense yellowish coloration in the acid medium but not in the alkaline medium.

TURBIDITY MEASUREMENT

Procedure. Exactly 0-, 1-, 2-, 3-, 4-, and 5-ml. portions of 100 p.p.m. of copper solution were transferred to test tubes. The volumes were adjusted to 5 ml. by the addition of water. Then 1 ml. of the complexing mixture was added to each test tube, and the solution was thoroughly mixed. Five drops of the ferrous sulfate solution were added without mixing. After 2 minutes, the solutions were mixed. After 5 minutes, the solutions were diluted to the 12.5-ml. mark and mixed again. The reference solution was made from 5 ml. of 0.01*M* copper sulfate solution by treating in the same manner. The relative turbidities were measured in the Fisher Nefluoro-Photometer. The tube at the far right was filled with the reference solution. The 430 filters were in the center and at the right, the blank filter at the left. A straight line was obtained when the turbidity readings of these solutions were plotted against copper concentration present in the solution. The turbidity readings (per cent transmittance) of 100, 84.0, 67.5, 48.5, 32.5, and 17.0 were obtained for 0.5, 0.4, 0.3, 0.2, 0.1, and 0.0 mg. of copper in 12.5 ml., respectively. A reading of 25% was obtained for 0.05 mg. of copper. The precision for the triplicate measurements was approximately 5% or better. The addition of gelatin solution showed an inhibiting effect on the formation of the precipitate. The copper(I) hydroxide precipitate was so fine that the use of protecting colloids was not necessary.

The effect of electrolytes on the properties of the precipitates has not been studied. However, the addition of sodium chloride and of magnesium nitrate affected the size and the physical properties of precipitates.

PROPERTIES OF PRECIPITATE

In the literature (*4*, *5*), the existence of cuprous hydroxide is described as questionable—and these authors prefer the name cuprous oxide ($CuO.xH_2O$). The copper(I) hydroxide precipitate obtained in the above procedure was stable at room temperature, but turned to dark red copper oxide by heating or by adding concentrated sulfuric acid. The precipitate was soluble in mineral acids, ammonium hydroxide, cyanide, and thiosulfate. When the precipitate was dissolved in dilute ammonium hydroxide, a purplish color of the cuprous test was obtained by shaking with biquinoline in isoamyl alcohol (*2*).

The pure precipitate could not be recovered quantitatively because it tended to be peptized by washing with water or dilute sodium hydroxide. Such peptization can be prevented considerably by washing with 1% magnesium sulfate solution which was adjusted to pH 10 with sodium hydroxide.

ACKNOWLEDGMENT

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Ceriometric Determination of Sugars

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Two procedures have been developed for determination of small changes in sugar concentration by ceric perchlorate oxidation. Both methods are applicable to estimation of semimicro quantities of sugars. Results obtained for several reducing sugars are given.

THE oxidimetric determination of glycerol, glucose, sucrose, and related compounds by means of the ceric perchlorate reagent was described by Smith and Duke (*1*, *2*). Two modifications of the procedure have been useful in investigating the kinetics of certain reactions involving small changes in the concentrations of various sugars. Results obtained with two pentoses and one triose are given, which extend the list of carbohydrate substances determined by oxidation with this reagent.

REAGENTS

Ceric perchlorate, approximately 0.28N in 4M perchloric acid. One liter of G. F. Smith Chemical Co. reagent-grade ceric perchlorate acid (in 6M perchloric acid) is mixed with 172 ml. of 72% perchloric acid. The resulting mixture is diluted to 2 liters with distilled water. This reagent is standardized against sodium oxalate or sodium arsenite and is stored in the absence of light.

Ceric perchlorate, 0.01 to 0.03N, in 2M perchloric acid. This solution is prepared by appropriate dilution of the above reagent. It is standardized daily against sodium oxalate or sodium arsenite and is also stored in the absence of light.

Sodium oxalate, 0.1800N, in 0.1M perchloric acid.

Sodium arsenite, 0.1300N. Accurately weighed arsenious oxide (approximately 13.0 grams) is dissolved in a mixture of 10 grams of anhydrous sodium carbonate and 1 liter of water. This solution is then diluted to 2 liters with water.

Osmic acid, 0.01M, in 0.1M sulfuric acid.

Nitro-ferroin indicator, 0.025M.

PROCEDURES

Method I. To 18 to 36 mg. of the sugar, in aqueous solution not exceeding 5 ml. in volume, add 20 ml. of the 0.28N ceric perchlorate reagent. Allow the oxidation to proceed at 25° C. for 1 hour, then add 25 ml. of the sodium oxalate solution to the mixture. Titrate the excess oxalate ion with the 0.03N ceric perchlorate reagent to a nitro-ferroin end point.

Method II. To 15 to 21 mg. of the sugar, in aqueous solution not exceeding 5 ml. in volume, add 10 ml. of the 0.28N ceric perchlorate reagent. After allowing the oxidation to proceed at 25° C. for 1 hour, add 2 drops of the osmic acid solution followed by 15.00 ml. of the arsenite solution to the oxidation mixture. Then titrate the excess arsenite with the 0.01N ceric perchlorate reagent to a nitro-ferroin end point.

DISCUSSION

Typical results are given in Table I. All calculations are based on consumption of 2n equivalents of cerate per mole of aldose, n being the number of carbon atoms in the sugar molecule. Among the sugars tested only DL-glyceraldehyde was incompletely oxidized in 1 hour. In Table II, a 4-hour oxidation period was required to achieve the theoretical consumption of oxidant for this substance.

Table I. Ceriometric Determination of Sugars

Sugar	Added, Mg.	Found, Mg.
	METHOD I	
D-Glucose	36.03	36.08 36.21 35.94 36.13 36.16
D-Xylose	36.03	35.85 35.91 36.02 35.82 36.13
D-Ribose	36.03	36.10 35.97 36.06
DL-Glyceraldehyde	36.03	35.91[a] 35.85[a]
	METHOD II	
D-Glucose	18.02	18.09 18.12 18.06

[a] Results for 4-hour oxidation.

The present procedures offer the advantages of sensitivity to small changes in sugar concentrations and titration to the disappearance of the indicator color rather than through a series of color changes as occurs in the reverse titration. These methods may be readily altered to accommodate different ranges of sugar concentration by appropriate changes in reagent concentrations.

Table II. Effect of Time on Ceric Perchlorate Oxidation of DL-Glyceraldehyde

Time, Hr.	Equivalents of Cerate Consumed per Mole of Sugar	Theory
1	5.62, 5.67, 5.66	
2	5.74, 5.72, 5.73	
3.75	5.98, 5.97	6.0

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Determination of Trace Amounts of Carbonyl Sulfide in Gaseous Hydrocarbons

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A simple method for determination of trace amounts of carbonyl sulfide in hydrocarbon gases is based on the reaction with piperidine in alcoholic solution to form piperidine oxythiocarbamate, which is determined spectrophotometrically. The method is applicable to gases containing hydrogen sulfide, sulfur dioxide, and aromatic hydrocarbons not exceeding the equivalent of 50 p.p.m. of benzene; higher concentrations of aromatics can be tolerated, provided they are known or can be determined. The results on synthetic blends are within the range of 2 to 75 p.p.m. of carbonyl sulfide and are reliable to within 5% of the amount present. A procedure is described for preparing carbonyl sulfide having a purity approaching 99.9%. The sulfide can be stored at 0° C. for several months in a stainless steel bomb without decomposition, provided its initial assay is better than 99.5%.

THE presence of carbonyl sulfide in gaseous hydrocarbons is an important problem to the petroleum industry, especially in processes where the catalysts employed are sulfur-sensitive and conventional methods for sulfur removal do not effectively remove carbonyl sulfide. Carbonyl sulfide has been speculated also to contribute to the corrosion of refinery equipment and may be responsible for the reduced efficiency of amine scrubbers (*3*). If the actual concentration of carbonyl sulfide could be determined in the gaseous products, then processes contributing to its formation could be studied either to remove more effectively the undesired constituent or to effect improvements in process design.

Numerous methods have been presented in the past few years for the determination of organic sulfur compounds in gaseous streams, but only a limited number are applicable to the determination of carbonyl sulfide. Avdeeva (*1*) described a quantitative method whereby the carbonyl sulfide was absorbed and oxidized in an ammoniacal calcium chloride solution, and then was determined by precipitating the sulfuric acid with barium chloride. Riesz and Wohlberg (*6*) proposed a method for the determination of carbonyl sulfide in hydrogen in which the constituent was absorbed with carbon disulfide in a piperidine-chlorobenzene reagent and the proportions were resolved colorimetrically. MacHattie and McNiven (*5*) and Hakewill and Rueck (*4*) have described methods whereby carbonyl sulfide can be determined by scrubbing gaseous samples with selective solvents and estimating the residual sulfur content of the gas by burning it, and then determining the oxides of sulfur which are formed. These methods are time-consuming and lack the sensitivity required for determining trace amounts of carbonyl sulfide in refinery gas streams.

The method for determination of carbonyl sulfide described by Brady (*2*), with certain modifications, has been found to be the most suitable from the standpoint of ease and rapidity with which it can be performed, and is not subject to interference from other sulfur-bearing constituents. The principle of the method is based on the observation that carbonyl sulfide can be quantitatively scrubbed from a gaseous sample as piperidine oxythiocarbamate which has a strong absorption band at 230 mμ.

The method presented is an adaptation of the Brady procedure and differs from it in several significant respects.

The apparatus has been modified to permit the determination of a few parts per million of carbonyl sulfide.

Synthetic standards are prepared for calibration purposes by blending gaseous hydrocarbons with measured quantities of pure carbonyl sulfide gas.

The interference of hydrogen sulfide and sulfur dioxide, constituents of many refinery streams, has been eliminated.

A procedure is described for the preparation and storage of pure carbonyl sulfide for long periods of time.

REAGENTS

Hydrocarbon Blending Gas. Any commercial grade of C_2, C_3, or C_4 hydrocarbon gas which is carbonyl sulfide–free.

Piperidine–Ethyl Alcohol Absorbent. The absorbent is prepared by dissolving 0.5 gram of piperidine in 1 liter of 95% ethyl alcohol and saturating the solution with the hydrocarbon blending gas. The reagent is stored in an amber-colored, glass-stoppered bottle.

Manganese Dioxide. This reagent must be specially prepared and is available from the Laboratory Equipment Co., Benton Harbor, Mich., Catalog No. 501-C.

Carbonyl Sulfide. The calibrating standard is prepared according to the procedure described. The purity of the standard should be ascertained just prior to use.

APPARATUS

A Beckman quartz spectrophotometer fitted with two calibrated 1-cm. quartz absorption cuvettes and equipped for ultraviolet spectroscopy was employed.

The gas handling apparatus, constructed of borosilicate glass, is illustrated diagrammatically in Figure 1.

The gas buret, *G*, is a 1-ml. Mohr pipet which is sealed to a capillary, 2-mm. bore T-stopcock. A suitable length of Tygon tubing connected to a 25-ml. gas leveling bulb, *L*, is fastened to the lower end of the pipet, with mercury being used as the confining liquid. Any leveling device may be used for raising and lowering the leveling bulb.

The reaction flask, *R*, consists of two 4-mm. bore stopcocks sealed diametrically onto a 5-liter capacity glass bulb. A 12/5 outer spherical glass joint is sealed to one stopcock and a 12/5 inner joint to the other. The blending flask, *B*, consists of a 5-liter flask with two 2-mm. bore stopcocks and a set of 12/2 spherical glass joints attached in the manner as the reaction bulb. The total volume of each bulb was determined to the nearest 5 ml.

The U-shaped manganese dioxide absorber, *A*, is a 50-cm. length of (6-mm. inside diameter) glass tubing, the ends being sealed to 12/5 outer spherical glass joints. The tube is nearly filled with manganese dioxide absorbent and the ends loosely plugged with glass wool.

The remaining portion of the manifold is

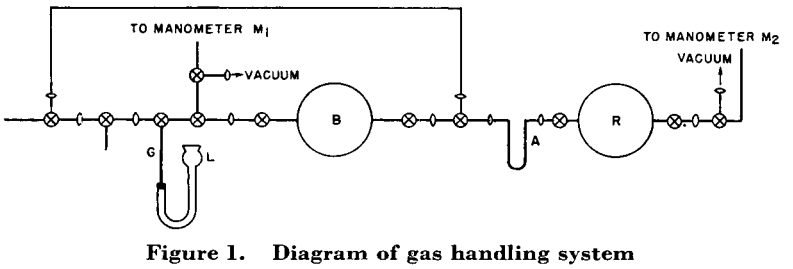

Figure 1. Diagram of gas handling system

made of 12/2 spherical glass joints and 2-mm. bore capillary glass tubing. All joints and stopcocks were lubricated with a silicone-type stopcock grease.

The dispensing tube (Figure 2) is a borosilicate glass tube closed on one end and fitted with a 12/5 inner spherical glass joint on the opposite end, and having a capacity not less than 50.5 ml. or greater than 51.0 ml., not including the bore of the glass joint.

EXPERIMENTAL

With the apparatus assembled as in Figure 1, the manifold was evacuated to the reaction flask and the system checked for leaks. The left-hand stopcock of the blending flask was closed, the evacuated portion of the manifold was filled with carbonyl sulfide, and approximately 0.9 ml. collected in the gas buret. The manifold was isolated from the buret, re-evacuated, and filled with about 0.85 ml. of carbonyl sulfide. The volume of gas remaining in the buret, the barometric pressure, and temperature at which the volume measurements were made were recorded. The carbonyl sulfide was transferred into the blending bulb with the hydrocarbon blending gas and the flask was filled to atmospheric pressure with the hydrocarbon gas. The reaction bulb and by-pass were evacuated and the manometer reading, M_2, was recorded. Approximately 100 mm. of gas pressure was admitted into the evacuated reaction bulb from the blending flask, at a rate not exceeding 250 ml. per minute, and the manometer reading again recorded. Blending gas was admitted by means of the bypass system until the pressure within the bulb was twice that of atmospheric pressure. (A pressure of 1 atmosphere in the reaction flask may be employed if concentrations of carbonyl sulfide between 2 and 4 p.p.m. are not of interest.)

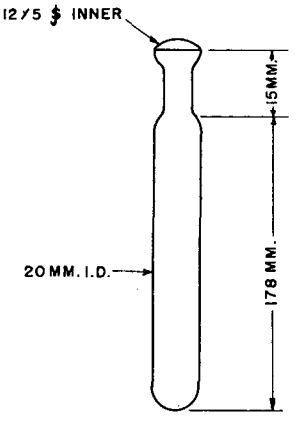

Figure 2. Dispensing tube

Fifty milliliters of the piperidine–ethyl alcohol solution were pipetted into the dispensing tube, the spherical joint was lubricated, and the vessel attached to the reaction flask by a spring clamp. The stopcock between the tube and bulb was opened, and the liquid displaced into the latter by shaking vertically. After the liquid had been transferred, the stopcock was closed, the tube detached from the flask, and the liquid in the flask shaken vigorously for 10 minutes. The lubricant was then completely removed from the inner spherical joint, including the bore, and the solution transferred into a 50-ml. flask. The flask was loosely stoppered and the contents allowed to stand for approximately 1 hour or until effervescence due to the dissolved gas had subsided.

Gas blends of increasing carbonyl sulfide concentration were prepared by introducing increments of approximately 160 and 275 mm. of gas pressure into the reaction flask. A blank solution was prepared as described, except the addition of carbonyl sulfide was omitted.

A portion of the piperidine oxythiocarbamate solution was transferred into a quartz absorption cell and the absorbance measured at 230 mμ against the prepared blank. The cuvette containing the blank solution was emptied, cleaned, dried, and refilled with a fresh portion of solution after each measurement. (It has been found advisable to work with only two cells and to complete the measurements as quickly as possible to avoid creep of the solution from the cuvette.)

The following formula was used to calculate the concentration of carbonyl sulfide added to the blending gas in the preparation of the calibrating standards.

$$\text{Carbonyl sulfide, milligrams} = \frac{273.1 \times 2.680 \times 10^{-3}(V)(P)}{760 \times 10^{-3}(T)}$$

where

V = volume of carbonyl sulfide added to blending flask, in milliliters
P = pressure introduced into the reaction flask from blending bulb, in millimeters of mercury
T = temperature in degrees Kelvin
2.680×10^{-3} = density of carbonyl sulfide at standard conditions

A calibration curve was prepared by plotting the measured absorbances against the milligrams of carbonyl sulfide in 50 ml. of solution. A typical calibration curve corrected for the blank values appears as a straight line with absorbance increasing as the milligrams of carbonyl sulfide increased.

Analysis of Samples. For applying the method to routine test samples, the portion of the manifold to the left of the manganese dioxide absorber was removed. The sample bomb was attached to the manifold and the system evacuated to less than 1-mm. mercury pressure and checked for leaks. The valve on the sample bomb was cautiously opened and the gas permitted to fill the evacuated system at a rate not exceeding 250 ml. per minute. The addition of gas was continued until the pressure of 2 atmospheres was attained. The reaction flask was removed from the system and the barometric pressure and the room temperature recorded. The piperidine–ethyl alcohol solution, 50 ml., was pipetted into the dispensing tube and the extraction performed as described; the blank is prepared from the carbonyl sulfide–free hydrocarbon blending gas.

A portion of the piperidine oxythiocarbamate solution was transferred into a quartz cuvette and the absorbance measured at 230 mμ against the prepared blank. The photometer reading was converted to the concentration of carbonyl sulfide in milligrams per 50 ml. using the prepared calibration curve.

The following formula was used to calculate the concentration on a weight basis in parts per million of carbonyl sulfide in the test sample.

$$\text{Carbonyl sulfide, p.p.m.} = \frac{760 \times 22{,}414 \times 10^3\,(C)(T)}{273.1\,(V)(P)(M)}$$

where

C = carbonyl sulfide concentration, in milligrams per 50 ml., corresponding to the measured absorbance
T = temperature in degrees Kelvin
V = volume of gas sample in milliliters
P = barometric pressure in millimeters of mercury, and
M = molecular weight of the sample.

Preparation of Carbonyl Sulfide. Pure carbonyl sulfide is not available commercially and must be specially prepared. The gas may be conveniently prepared, however, from the reaction of potassium thiocyanate and sulfuric acid, then purified by passage over Ascarite in combination with repeated low-temperature fractionation.

The apparatus (Figure 3) consists essentially of two parts, the portion to the left of the U-tube condenser being the gas prepara-

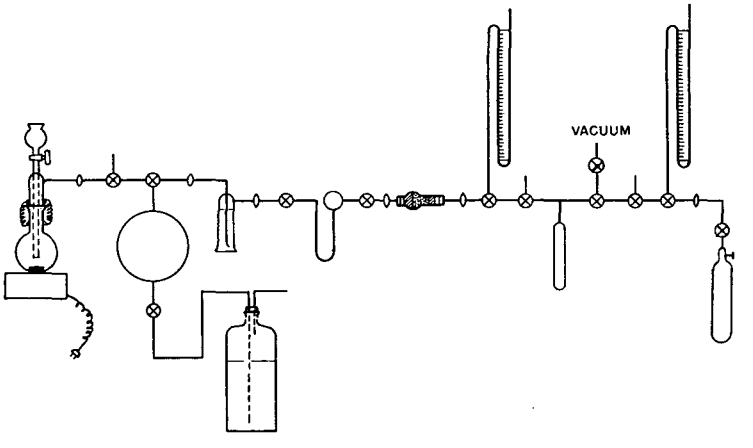

Figure 3. Diagram of apparatus for preparation of carbonyl sulfide

Table I. Analysis of Gas Blends for Carbonyl Sulfide

Blend No.	Carbonyl Sulfide, P.P.M. Found	Added	Difference
1	2.0	2.1	−0.1
2	3.9	4.0	−0.1
3	5.5	5.3	+0.2
4	23.5	22.6	+0.9
5	23.4	23.2	+0.2
6	25.8	25.0	+0.8
7	33.0	33.7	−0.7
8	36.9	36.2	+0.7
9	48.0	49.0	−1.0
10	59.2	60.0	−0.8

Standard deviation, 3.1%.

tion system and that to the right the purification and fractionation system. All components of the gas manifold are fabricated from borosilicate glass.

The gas generator consists of a 500-ml. flat-bottomed flask fitted with a 24/40 outer tapered glass joint. The inner portion of the glass joint is sealed to the stem of a 100-ml. separatory funnel and carries a side arm sealed to a 12/5 outer spherical glass joint for subsequent attachment to the manifold. Stirring of the reaction mixture is accomplished with a magnetic stirrer.

The brine bulb is fabricated from a capillary, 2-mm. bore T-stopcock and a 4-mm. bore stopcock, sealed diametrically to a 5-liter bulb. The bulb is connected to a 2-gallon siphon bottle containing 6 to 7 liters of acidified water saturated with sodium chloride.

The gas scrubber is a conventional 125-ml. capacity gas washing bottle fitted with a coarse porosity fritted-glass disk and filled with 150 ml. of 30% aqueous potassium hydroxide solution.

The purification side of the system consists of a U-tube condenser conforming to the dimensions shown in Figure 4 and a single bulb absorption tube sealed to a 12/2 inner spherical glass joint, containing Ascarite and stoppered at both ends with glass wool. Two open-end manometers, a cold finger manifold (Figure 5), and three 1-quart capacity Dewar flasks complete the unit.

The pure carbonyl sulfide is collected in a 150-ml. capacity stainless steel bomb having a working pressure of 1800 pounds per square inch when fitted with a stainless steel needle valve. The bomb and valve sold by Hoke Inc., Englewood, N. J., Catalog No. HS-150 and 343, respectively, are satisfactory for this purpose. A safety shield is recommended for use in the carbonyl sulfide purification techniques.

The gas generator is disassembled and a glass- or plastic-encased stirring bar is inserted into the flask followed by 400 ml. of 55% by volume sulfuric acid. The male portion of the tapered joint is lubricated and the flask is attached and fastened to it with springs. The magnetic stirrer is turned on, the separatory funnel stopcock closed, and 40 ml. of a saturated aqueous solution of potassium thiocyanate are introduced into the funnel. Approximately 10 ml. of the thiocyanate solution are added to the flask and the generator is flushed with gas for 10 minutes to an exhaust hood. The generated gas is then collected in the brine bulb by displacement; additional portions of the thiocyanate solution are introduced periodically as required.

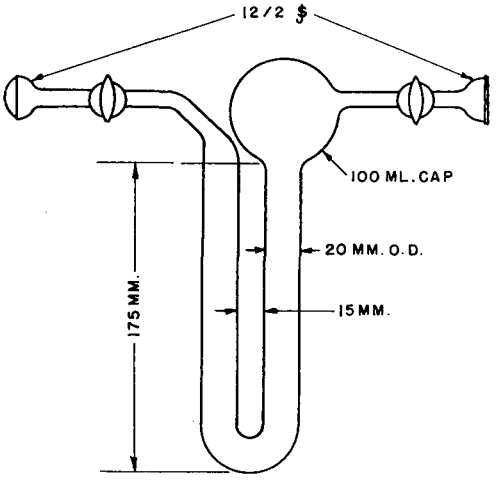

Figure 4. U-tube condenser

The brine bulb, filled with impure carbonyl sulfide, is closed off from the apparatus and the latter is evacuated to less than 1-mm. mercury pressure and checked for leaks. A Dewar flask containing liquid nitrogen is placed around the U-tube condenser and the gas in the brine bulb displaced through the potassium hydroxide scrubber as rapidly as possible, collecting the condensable fraction in the U-tube condenser. The flow rate for this operation should be 750 to 1000 ml. per minute; a slower rate results in the absorption of excessive amounts of carbonyl sulfide. The U-tube is evacuated periodically, during the condensation process, to pump out noncondensables. Approximately 5 cm. of the cold finger are immersed in liquid nitrogen, the Dewar flask is removed from around the U-tube condenser, and the liquid allowed to vaporize over the Ascarite and condense in the cold finger. (The coating of frost which forms on the outside of the U-tube condenser tends to control the rate of vaporization and should not be removed.) When all of the liquid has been transferred, a white powdery residue should remain in the U-tube. The U-tube condenser is then evacuated until all of this volatile residue has been pumped out and the pressure has been reduced to less than 1-mm. mercury pressure. A small section of the U-tube is immersed in liquid nitrogen and the liquid in the cold finger is allowed to vaporize over the Ascarite and recondense in the U-tube condenser. This process is continued until only a white powdery residue remains in the cold finger. The needle valve on the carbonyl sulfide bomb is opened and the system evacuated until the volatile residue in the cold finger is completely removed. The cold finger is reimmersed in liquid nitrogen, and the liquid allowed to vaporize over the Ascarite and recondense in the cold finger. The system is then evacuated to less than 1 mm. of mercury pressure for 5 minutes.

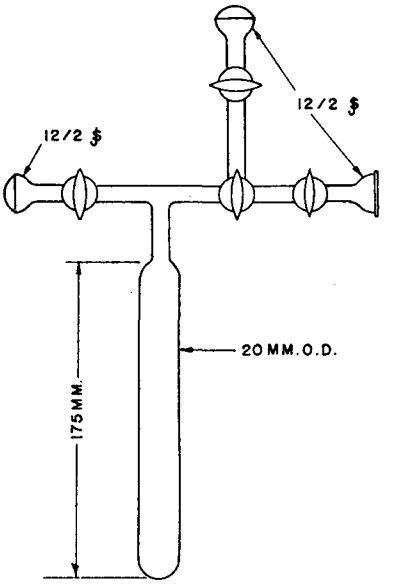

Figure 5. Cold finger manifold

Approximately one third of the carbonyl sulfide bomb is immersed in liquid nitrogen. The purified carbonyl sulfide is vaporized from the cold finger and condensed in the evacuated sample bomb until approximately one tenth of the original liquid volume remains in the cold finger. The bomb is then evacuated to less than 1-mm. mercury pressure for 5 minutes, the needle valve is closed, and the cylinder is removed from the liquid nitrogen and stored at 0° C. With experience, carbonyl sulfide prepared in this manner should assay 99.8 to 99.9 mole %.

Carbonyl sulfide may be assayed for impurities by a mass spectrometer using techniques normally employed in determining purity. Carbonyl sulfide prepared in the manner described usually does not contain acid gases and the amount of carbon disulfide present is generally too small to cause interference; hence, the carbonyl sulfide may be used directly to establish a calibration.

DISCUSSION

The main objective was to develop and to evaluate a method for the determination of carbonyl sulfide, particularly as applied to the relatively low concentrations usually found in refinery hydrocarbon gas streams. After the procedure had been demonstrated to show promise, a series of gas samples was prepared from ethylene, nitrogen, sulfur dioxide, and hydrogen sulfide containing known amounts of carbonyl sulfide. The analytical results shown in Table I demonstrate the applicability of the

method with such a sample type and represent analyses obtained with two different lots of carbonyl sulfide gas.

The method has been applied with equal success on a control basis to the analysis of ethylene produced in commercial quantities. In general, an analysis can be completed in 2 hours, including the time required for preparation of the reference, or blank solution, and for the effervescence due to dissolved gas to subside.

Effect of Carbonyl Sulfide Concentration. The relation between the absorbance of the piperidine oxythiocarbamate complex and the concentration of carbonyl sulfide is illustrated by a straight line. Within the limits, 0.0 to 0.7 mg. of carbonyl sulfide per 50 ml. of solution, the absorbance of the complex follows Beer's law. The minimum concentration which can be determined reliably with a 1-cm. light path cell is approximately 0.05 mg. of carbonyl sulfide in 50 ml. of reagent.

Effect of Time on Standing. A series of gas samples was prepared containing known amounts of carbonyl sulfide. These samples together with the blending gas were processed according to the procedure. Absorbance values taken at hourly intervals indicated that the measurement may be made within an 8-hour period (Table II). The time required for effervescence of the dissolved gas may vary somewhat with temperature and with the solubility of the gas in ethanol; however, 1 hour is usually sufficient.

Table II. Stability of Piperidine Oxythiocarbamate Solution

Blend No.	Absorbance, 1-Cm. Cell		
	1 hr.	4 hr.	8 hr.
1	0.237	0.240	0.239
2	0.260	0.263	0.263
3	0.268	0.269	0.269

During the early part of the investigation, a shift in the calibration curve occurred on successive days. This was ultimately traced to the reference solution creeping out of the cell and over the outer surface of the cuvette windows when multiple absorbance measurements were made. For this reason, only two cuvettes are recommended to be employed, preferably of a glass-stoppered type, and the measurements should be completed in the minimum length of time. If the measured values of the blanks, when compared to the original reagent, deviate by an amount greater than that equivalent to 1% transmittance, the blank determination should be repeated until this precision of measurement can be achieved.

Interference. This procedure was designed to function primarily as a control on the amount of carbonyl sulfide contamination. Refinery gas streams contain other sulfur-bearing compounds that react with the reagent or interfere in some other manner. These diverse compounds are believed to be predominantly hydrogen sulfide and sulfur dioxide with smaller amounts of thiophene, carbon disulfide, and possibly methanethiol. If the interference of hydrogen sulfide and sulfur dioxide could be eliminated simply, or reduced to a nominal level through the use of a suitable absorbent, the method would have greater flexibility. Therefore, a study was made to determine what effect various types of absorbents for these diverse constituents might have on carbonyl sulfide. The two absorbents selected first were asbestos fibers impregnated with lead acetate, and manganese dioxide. The experiment consisted of blending hydrocarbon gases with known amounts of carbonyl sulfide, hydrogen sulfide, and sulfur dioxide and passing the mixtures over the absorbent at various flow rates. Tests demonstrated that lead acetate completely absorbed hydrogen sulfide and that only a minor portion of the sulfur dioxide was retained. When blends of similar composition were passed over manganese dioxide, however, the contaminating gases were removed completely and the carbonyl sulfide was recovered *quantitatively*. A limited number of experimental results selected at random have been tabulated in Table III. These data substantiate the statement that hydrogen sulfide and sulfur dioxide interference is eliminated without affecting the validity of the analytical result for carbonyl sulfide by passing the sample over manganese dioxide prior to the formation of the carbamate complex.

Table III. Removal of Hydrogen Sulfide and Sulfur Dioxide by Manganese Dioxide

Added, P.P.M.		Carbonyl Sulfide, P.P.M.	
Hydrogen sulfide	Sulfur dioxide	Added	Found
..	..	25.0	25.2
..	..	24.2	24.2
20.3	..	24.9	24.8
21.6	..	24.1	24.6
20.2	..	24.3	24.0
59.7	..	23.6	23.4
20.2	26.1	24.1	24.4
18.0	21.1	24.8	24.5

Standard deviation, 1.2%.

Aromatic hydrocarbons, such as benzene, in concentrations not exceeding 50 p.p.m. do not appreciably interfere in the method. The correlated data in Table IV indicate that a blend containing 56 p.p.m. of aromatics, such as benzene or toluene, has an absorption which is equivalent to about 1 p.p.m. of carbonyl sulfide, an error which can be disregarded except when the method is extended to its lower concentration limit. For application of the method to samples containing aromatics in excess of the concentration mentioned, the sample may first be scrubbed with 95% ethyl alcohol and the transmittance of the alcoholic solution measured at 255 mμ against an ethanol blank for correction of the absorbance owing to the aromatic compounds. Interference from thiophene and carbon disulfide can be compensated by measurement of the absorbance of the piperidine-alcohol scrubbing solution at 230, 240, and 290 mμ and application of standard principles to the analysis of a three-component system (*2*).

Table IV. Effect of Aromatic Hydrocarbons

Aromatic	Concn., P.P.M.	Equivalence as Carbonyl Sulfide, P.P.M.
Benzene	56	1.0
	560	7.5
Toluene	56	0.5
	560	6.0

The grease should be removed from the inner spherical glass joint of the reaction bulb prior to transfer of the ethyl alcohol solution into a receiver. All silicone-type stopcock lubricants tested exhibited absorption in the vicinity of 230 mμ and hence would lead to high results for carbonyl sulfide if dissolved in the ethyl alcohol–piperidine reagent. A minimum amount of the lubricant should be employed to seal the inner joint, as well as its connecting stopcock, and particular care exercised to preclude subsequent contamination of the alcoholic solution.

Contamination and Storage of Carbonyl Sulfide. Carbonyl sulfide produced from the reaction of sulfuric acid with potassium thiocyanate is contaminated with hydrogen sulfide, sulfur dioxide, carbon dioxide, carbon disulfide, oxygen, and nitrogen. To obtain carbonyl sulfide of sufficient purity for calibration purposes, a purification technique for the removal of these contaminants was developed so that a sufficient yield of carbonyl sulfide could be obtained conveniently for subsequent blending purposes. Aqueous potassium hydroxide and Ascarite are excellent absorb-

ents for acid gases but also absorbed exorbitant amounts of carbonyl sulfide. Therefore, the generated gas is passed as rapidly as practical through the caustic scrubber as well as the Ascarite-filled absorption tube. Repeated scrubbing of the impure gas over Ascarite will effectively remove the remaining traces of hydrogen sulfide, sulfur dioxide, and carbon dioxide.

Various methods were tested for removing the carbon disulfide, repeated low temperature fractionation being most successful. Evacuation of the product at liquid nitrogen temperature eliminates the remaining undesirables, oxygen and nitrogen, and materially shortens the time for purification without introducing subsequent variable factors. With this preparation procedure, approximately 1 gram of pure carbonyl sulfide should be obtained.

Carbonyl sulfide can be stored for several months at 0° C. in a stainless steel bomb fitted with a stainless steel valve. In the early stages of the work, a stainless steel bomb fitted with a brass needle valve and connector was employed. Over a short period of time the composition of the gas had changed appreciably, the decomposition apparently being catalyzed by the brass.

Accuracy. The reliability of the method is illustrated by the data in Tables I and III. Analyses performed for the purpose of checking the performance of the method or evaluating various lots of freshly prepared carbonyl sulfide indicated a maximum absolute deviation of 1 p.p.m. from the theoretical value within the range of 2 to 60 p.p.m., or 3.1% when expressed as an estimated standard deviation.

ACKNOWLEDGMENT

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Determination of Lead in Urine

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A method is described for the determination of lead in urine which eliminates the necessity for time-consuming precipitation and ashing. Lead, as lead iodide, is extracted quantitatively from acid solution with methyl isopropyl ketone. The lead is then removed from the ketone layer with an aqueous sodium hydroxide solution and is determined colorimetrically by the dithizone method of Snyder using the lead-bismuth separation of Bambach and Burkey.

THE detection of lead poisoning is greatly facilitated by the determination of micro quantities of lead in the urine of the individual (*2, 6, 10*). The majority of methods currently in use (photometric, polarographic, etc.) (*1, 3–5, 8, 11*) involve precipitation of the lead as the phosphate, and usually ashing, for the removal of various organic and inorganic constituents which are present in relatively large amounts in urine. In the experience of this laboratory, the initial precipitation of the lead as the phosphate in alkaline solution, with calcium and other ions, is the tedious and time-consuming part of the analytical procedure, especially when larger volumes of urine must be used when amounts of lead present are small.

The introduction of methyl isopropyl ketone as an organic solvent for the quantitative extraction of lead as lead iodide by West and Carlton (*12*) furnishes a means for considerably reducing the time required for an analysis. In the proposed method, lead, in an acid solution and in the presence of excess potassium iodide, is extracted quantitatively by methyl isopropyl ketone. This is followed by extraction from the ketone into basic solution and development of color with dithizone. The dithizone method is that of Snyder (*9*) using the lead-bismuth separation of Bambach and Burkey (*3*).

REAGENTS AND APPARATUS

A blank must be run to determine the amount of lead in the reagents and distilled water supply before their use. If the total amount is more than 0.2 γ, the reagents must be purified. All chemicals should be reagent grade.

Nitric acid, concentrated.

Diethyl ether.

Methyl isopropyl ketone, Eastman No. 3146.

Potassium iodide. Dissolve 155 grams of potassium iodide in 100 ml. of distilled water. This includes a slight excess to ensure saturation.

Sodium hydroxide, 0.5%. Dissolve 0.5 gram of sodium hydroxide in 100 ml. of distilled water.

Thymol blue indicator, 0.1%. Dissolve 0.1 gram of thymol blue WS in 100 ml. of distilled water.

Ammonium citrate, 5%. Dissolve 5 grams of ammonium citrate in 100 ml. of distilled water.

Sodium cyanide, 2%. Dissolve 2 grams of sodium cyanide in 100 ml. of distilled water.

Ammonium hydroxide, 14%. Mix equal volumes of concentrated ammonium hydroxide and distilled water.

Dithizone solution. Dissolve 25 mg. of dithizone (Eastman No. 3092) in 1 liter of chloroform. Store in a glass-stoppered bottle in the refrigerator.

Buffer, pH 3.4. Dilute 9.1 ml. of concentrated nitric acid to approximately 500 ml. with distilled water. Adjust the pH to 3.4 with ammonium hydroxide. Add to this solution a mixture of 25 ml. of 0.2*M* potassium acid phthalate and 4.98 ml. of 0.2*M* hydrochloric acid. Dilute to 1 liter with distilled water.

Ammoniacal cyanide solution. Mix 5 volumes of 14% ammonium hydroxide, 1 volume of 1% sodium sulfite, and 1 volume of 2% sodium cyanide. [The sodium sulfite is added to reduce oxidants which attack the dithizone (*7*).]

Standard lead solution. Dissolve 0.160 gram of lead nitrate in 1 liter of 1% nitric acid. Dilute further as needed with 1% nitric acid.

All traces of lead must be removed from the apparatus by rinsing with warm dilute nitric acid and distilled water, then shaking with ammoniacal cyanide solution and dithizone solution until no pink color is seen in the chloroform layer. Finally, rinse once with distilled water.

PREPARATION OF SAMPLE

Urine. To 100 ml. of urine in a 500-ml. Florence flask, add 10 ml. of concentrated nitric acid and reflux for 20 minutes. Cool under tap water and transfer to a 250-ml. separatory funnel, rinsing the flask with warm dilute nitric acid. Extract with 25-ml. portions of diethyl ether three times, discarding the ether layers.

PROCEDURE

To the prepared sample in a 250-ml. separatory funnel, add 10 ml. of saturated potassium iodide solution and extract three times with 10-ml. portions of methyl isopropyl ketone saturated with 5% hydrochloric acid. Combine and save the ketone layers in another separatory funnel. Remove the lead from the ketone by extracting with 50 ml. of 0.5% sodium hydroxide. Discard the ketone layers. Add 4 drops of indicator to the sodium hydroxide solution. Adjust the pH of this solution to the acid side of the alkaline range of the indicator (pH 9.5 to 10.0) with nitric acid. Add 10 ml. of 5% ammonium citrate and 10 ml. of 2% sodium cyanide. Readjust the pH to 9.5 to 10.0, if necessary, with ammonium hydroxide.

Shake with 20-ml. portions of dithizone solution until the green color of the dithizone remains unchanged, saving the chloroform layers in a separatory funnel containing 25 ml. of a buffer solution, pH 3.4. (Each 20-ml. portion of dithizone solution represents 187 γ of lead.) Shake the separatory funnel containing dithizone solution and buffer for 1 minute. Discard the chloroform layer. If more than 187 γ of lead are present, use aliquots, diluting with buffer solution (pH 3.4) to keep the pH unchanged. Add 75 ml. of ammoniacal cyanide solution to bring the pH to 11.5, add 25 ml. of dithizone solution, and shake for 1 minute. Filter the red chloroform layer containing lead dithizonate into a clean dry colorimeter tube.

Carry a blank consisting of 25 ml. of 1% nitric acid through the procedure and adjust it to 100% transmittance at a wave length of 510 mμ. Note absorbance of the unknown, and, by referring to a standard curve, obtain the concentration of lead.

RESULTS AND DISCUSSION

In Table I, the results are listed for analyses of known concentrations of lead in urine using a Coleman Universal spectrophotometer and a 22-mm. cell.

Extraction of lead from acid solution by methyl isopropyl ketone presents several specific advantages. In the extraction of lead with dithizone-chloroform a clear ammoniacal solution is necessary. In the presence of many alkaline precipitates (calcium and magnesium phosphates, ferric hydroxide, etc.) lead may be occluded and troublesome emulsions may be formed. Some materials, such as urine, contain more of these substances than can be kept in solution with any reasonable amount of citric acid (*1*). The extraction of lead in acid solution with methyl isopropyl ketone accomplishes the initial isolation and concentration of the lead while avoiding the tedious precipitation, entrainment, and ashing necessary in some of the other methods and avoiding the formation of emulsions due to alkaline precipitates. An important advantage also is the ready adaptability of this method to larger volumes of urine.

Furthermore, the time required for each analysis is reduced from hours to approximately 45 minutes. By using several extractions with methyl isopropyl ketone, lead may be quantitatively removed without the waiting period of 1 hour recommended by West (*12*) for the complete separation of the ketone layers. Troublesome emulsions with methyl isopropyl ketone may occur in the analysis of urine but may be avoided by a preliminary acid hydrolysis and ether extraction of the urine.

In the presence of sodium or potassium cyanide interfering ions are limited to stannous tin, thallium, and bismuth (*1*). Stannous tin and thallium are rarely encountered in urine and may usually be ignored. The bismuth may be removed by a preliminary acid-thiocyanate extraction (*12*) or by an acid chloroform-dithizone extraction (*3*), in which, at a pH of 3.4, the lead is extracted into the aqueous layer, while bismuth remains combined with dithizone in the chloroform layer and is discarded. This pH is critical. At a higher pH, lead will remain in the chloroform layer, giving low results; at a lower pH, bismuth will also be extracted into the buffer, giving high results.

Table I. Recovery of Lead from Urine

Lead, γ		
Added	Found	Difference
0.0	0.1	+0.1
1.0	1.1	+0.1
2.0	2.0	0
5.0	4.7	−0.3
10.0	10.2	+0.2
10.0	10.2	+0.2
10.0	9.7	−0.3
15.0	15.8	+0.8
15.0	15.3	+0.3
15.0	14.4	−0.6
20.0	20.0	0
20.0	19.5	−0.5
25.0	24.9	−0.1
30.0	29.2	−0.8
30.0	29.8	−0.2
35.0	34.7	−0.3

Standard deviation = 0.39.

By extracting an aqueous solution of lead with dithizone solution at a pH of 11.5, the lead as lead dithizonate is quantitatively transferred to the chloroform layer and the excess dithizone is concentrated in the aqueous layer presumably as the ammonium salt (*9*). This removal of excess dithizone results in a low, but constant, concentration of unreacted dithizone in the chloroform layer, thus improving the accuracy and reliability of the results as compared with the lower pH methods. This high pH extraction also permits the use of a standard dithizone solution and eliminates the necessity for titrimetric extraction to estimate concentration of lead in the sample. The preliminary extraction at a pH of 9.5 to 10.0 enables the analyst to make a rough estimate of the amount of lead present.

The range of sensitivity of the method, 0 to 70 γ using a 22-mm. cell and a Coleman Universal spectrophotometer, is sufficient to determine the microquantities of lead found in mild, chronic lead poisoning which would otherwise be difficult to detect. By varying cell size the range may be increased (*9*).

It is absolutely essential that the last traces of lead be removed from all equipment used in the analysis.

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Standardization of Sulfuric Acid against Sodium Carbonate

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Sulfuric acid, 1*N*, has proved to be a satisfactory reference standard. Sodium carbonate is a reliable and convenient acidimetric standard. The difference between six commercial sodium carbonates is less than 1 part in 1000.

IN ORDER to achieve a highly precise standardization of a 1*N* sulfuric acid solution that has been in use in this laboratory as a reference standard since 1944, the acid was titrated in 1951 against each of six of the best sodium carbonates available commercially.

EXPERIMENTAL

Six different sodium carbonates of the highest quality obtainable were bought for this work. A portion of each, taken from a newly opened bottle, was heated in a weighing bottle to constant weight at 250° C., and stored in a desiccator. On the day of titration for a given carbonate, 5 grams were taken from the weighing bottle and used in the preparation by weight of about 100 grams of aqueous solution. Five separate 10-gram portions of this solution were then compared with the reference standard, 1*N* sulfuric acid.

One drop of 0.1% methyl orange was added to the weighed portion of carbonate solution to serve as preliminary indicator. The reference standard was then added from a weight buret to pH 3, this end point being determined by visual comparison to a standard buffer. After carbon dioxide had been expelled by boiling, and the flask washed down with freshly boiled distilled water, 2 drops of 0.1% bromothymol blue were added and the titration was finished volumetrically with dilute sodium hydroxide.

Each carbonate solution was titrated completely in one day to reduce any risk of errors due to chemical changes.

The mixed indicator gives a satisfactory warning that the end point is being approached, and a sharp end point at pH 7. No end point correction was applied.

Calculation employing the dissociation of bisulfate ion shows that the presence of this ion causes the results to be low by less than 1 part in 100,000.

The precision of the titrations was improved measurably by adding the sulfuric acid to pH 3 in all titrations. Complete expulsion of carbon dioxide by boiling is certain at this pH. Several preliminary sets of data in which the pH at this stage was allowed to vary were comparatively erratic. This experience indicates the control to within about ±0.2 unit of pH 3 is desirable.

RESULTS

The experimental results, all corrected to vacuum, are given in Table I. The grand mean for the table is $\overline{\overline{X}} = 48.679$ mg. of sodium carbonate per gram of reference standard, the unit used throughout Table I.

In Table I, X_1 to X_5 are the results of individual titrations, $\overline{X}$ is the mean for each carbonate, and the standard deviation is

$$s = \sqrt{\frac{\Sigma_i(X_i - \overline{X})^2}{n - 1}} \qquad (1)$$

PRECISION OF TITRATIONS

The precision attained in the titration of any one carbonate is indicated by the corresponding standard deviation, s. A conservative estimate of the reliability of any single titration is $3s$ (*2*). The worst case in Table I is carbonate 3, where $3s$ is 0.04 or 1 part in 1200 for a single titration. The reliability of the mean of the five titrations for each carbonate is improved by a factor of 2.24, since $s_m = \frac{s}{\sqrt{n}}$. Thus, the reliability of the mean becomes 1 part in 2700 in the worst case (carbonate 3) and is 1 part in 8000 in the best cases (carbonates 1 and 4).

RELIABILITY OF SODIUM CARBONATE

The standard deviations,

$$s' = \sqrt{\frac{\Sigma_i(\overline{X}_i - \overline{\overline{X}})^2}{(n - 1)}} \qquad (2)$$

for the six $\overline{X}$ values in Table I may be taken to measure the over-all reliability of sodium carbonate as an acidimetric standard. The value of s' is 0.3 part per 1000 and $3s'$ is 0.9 part per 1000. The conclusion is that the strength of sulfuric acid standardized as described by five replicate titrations against a high quality sodium carbonate may be guaranteed (*2*) to 1 part per 1000.

Inasmuch as the sulfuric acid was standardized against six sodium carbonates of high quality, its strength can be guaranteed accordingly to $0.9/\sqrt{6}$, or 0.4 part per 1000.

Table I. Summary of Titration Results

Carbonate	1	2	3	4	5	6
X_1	48.667	48.662	48.703	48.693	48.689	48.670
X_2	48.658	48.660	48.679	48.693	48.694	48.679
X_3	48.659	48.654	48.708	48.690	48.684	48.685
X_4	48.663	48.652	48.688	48.682	48.678	48.681
X_5	48.668	48.664	48.708	48.692	48.674	48.686
$\overline{X}$	48.663	48.658	48.697	48.690	48.684	48.680
s	0.0045	0.0052	0.0131	0.0045	0.0081	0.0064

Carmody (*1*) used a titration against standard hydrochloric acid approximately 0.5*N* to measure the purity of his carbonates but distilled only two batches of acid. The reliability of constant-boiling hydrochloric acid as a standard has been discussed previously (*2*, *3*).

The sulfuric acid was standardized against 14 batches of constant-boiling acid over a 3-year period. The average normality obtained for the reference acid agreed with that from all the data in Table I to within less than 1 part in 25,000. This agreement is one order of magnitude better than the foregoing guarantees.

The data of Table I suggest that measurable differences (less than one part in 1000) in equivalent weight exist among the six sodium carbonates used. Variance analysis of the results from an experimental design providing for complete randomization should reveal any such differences.

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Interferences in the Titanium Sulfate Method for Hydrogen Peroxide

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The usual procedure of using the acidic titanium sulfate method for determining hydrogen peroxide will give erroneously low values in the presence of formaldehyde because of the reactions between these two species in solution. Other oxygenated organic species may possibly also cause interferences. A modified procedure, which was tested on mixtures of hydrogen peroxide with each of a number of possible interfering substances, was found to give reliable results with all mixtures studied and is recommended as the preferred method.

THE intense yellow color formed when an acidic solution of a titanium salt reacts with hydrogen peroxide is a sensitive test, reported (*3*, *7*) to be specific to hydrogen peroxide or to related compounds, such as inorganic peroxides, which will form hydrogen peroxide during the analytical procedure. If the color intensity is measured in a colorimeter or spectrophotometer, the method can be used for quantitative determination. However, no information has been published on possible interferences with this method. There have been several recent instances of its use in the analysis of products formed in the partial oxidation of hydrocarbons. The difference between the total peroxide content as determined, for example, by iodide titration and that indicated by the titanium sulfate method was attributed to organic hydroperoxides formed in the reaction. The extent to which organic hydroperoxides or hydrogen peroxide or both are in fact formed is a significant question in various kinetic studies. This study, therefore, considered the possibility of interferences on the usual titanium sulfate method from various other compounds which may be formed or are representative of those formed in hydrocarbon oxidations, and a modification of this method is recommended.

MODIFIED PROCEDURE

Prepare a reagent solution by adding 10 grams of titanium sulfate and 20 grams of concentrated sulfuric acid to 50 ml. of distilled water. Allow the mixture to stand for 24 hours, then decant and filter on analytical filter paper to remove suspended solids. Dilute the test solution with sulfuric acid of such concentration that the diluted solution is about $3N$ and allow to stand for 2 hours. Add 1 ml. of the reagent solution to 10 ml. of the diluted test solution and determine the absorbance of the yellow color (at a wave length of 420 mμ) developed in comparison with a blank containing 10 ml. of distilled water and 1 ml. of the titanium sulfate solution. Compare this with a calibration curve determined from known concentrations of hydrogen peroxide.

For the present studies a Lumetron colorimeter with a 420 mμ filter was used. The meter is accurate to within about ±2%.

The essential difference between this modified procedure and the usual procedure (*1*, *3*, *5*) is that here the test solution is diluted with acid instead of water and it is allowed to stand before continuing the procedure. Other procedures (*2*, *4*, *6*) also differ from the above in the composition of the titanium reagent solution and ratio used of this solution to the test solution, but within fairly broad limits these latter differences appear to be relatively unimportant.

In studying the effects of possible interfering compounds, a mixture of the organic species and hydrogen peroxide containing 70 to 130 grams per liter of the latter was prepared and allowed to stand for about 16 hours before analysis in order to provide opportunity for possible reaction. At the end of the 16-hour period the peroxide content was determined by iodide analysis, taking care to avoid air oxidation, and then the sample was diluted and subjected to the titanium sulfate analysis method. For mixtures of acetaldehyde in mole ratios to hydrogen peroxide of 5 to 1 and 2 to 1 and for methanol in a mole ratio of 5 to 1, color development with the usual unmodified procedure was instantaneous, and the hydrogen peroxide found was within ±3% of the true value. However, for formaldehyde, in a mole ratio of 2 to 1, the initial color development corresponded to only about 40 to 45% of the peroxide present, or to about 80 to 85% if the mole ratio were 1 to 1. The transmittance slowly increased over several hours, after 5 hours becoming equal to 105 to 110% of that for the true value of the peroxide present. There were indications that formic acid might also cause some interference, but this was not pursued with the original procedure.

With the modified procedure, full color development is instantaneous, the color is stable for at least several hours, and the results were found to be within ±3% of the true value in the presence of all species studied for possible interference, as follows:

Species Studied	Concentration Ratios Studied, Mole Ratio of Organic Species to H_2O_2
Formaldehyde	4:1, 2:1, 1:1, 1:2
Acetaldehyde	5:1
Formic acid	8:1, 4:1, 2:1
Acetic acid	5:1
Acetone	5:1
Methanol	5:1
Crotonaldehyde	5:1
Methyl ethyl ether	5:1

There is no significant difference in the results if the diluted solution is allowed to stand for the specified time (2 hours) at an acid concentration as low as $0.5N$.

The modified and unmodified procedures were also tested on *tert*-butyl hydroperoxide alone and on its mixtures with hydrogen peroxide, using acetic acid–water as the solvent. Only the hydrogen peroxide was detected; the absorptivity of the *tert*-butyl hydroperoxide alone is about $^1/_{500}$ of that of an equivalent amount of hydrogen peroxide. MacNevin and Urone (*8*) have recently reported that mixtures of hydrogen peroxide and various hydroperoxides could be separated in polarographic analysis by complexing the hydrogen peroxide with titanium ion. This result plus the above observation suggest that a suitable colorimetric procedure can be used for determining hydrogen peroxide in the presence of organic hydroperoxides. It would be reassuring, however, if this were confirmed on some of the low molecular weight hydroperoxides such as methyl and ethyl hydroperoxide.

DISCUSSION

The low results obtained upon using the unmodified procedure on mixtures of formaldehyde and hydrogen peroxide are undoubtedly caused by the fact that these substances react to form hydroxy alkyl peroxides:

$$HCHO + H_2O_2 \rightleftharpoons HCH(OH)OOH \quad (1)$$

$$HCHO + HCH(OH)OOH \rightleftharpoons HCH(OH)OOCH(OH)H \quad (2)$$

The reactions are reversible and acid-catalyzed in both directions. More information concerning equilibria and kinetics and direction to earlier literature is given in a recent paper (*9*). The principle underlying the modified procedure suggested here is to cause reversal of these two reactions to occur before the titanium reagent is added. The present studies suggest that the titanium reagent forms a colored complex with the hydroxy-

methyl hydroperoxide but not with the dihydroxydimethyl peroxide, and furthermore that the complex with hydroxymethyl hydroperoxide may have a slightly greater absorbancy than that formed with hydrogen peroxide.

There are several reasons for this conclusion: The dilution of a test sample for analysis will cause Reaction 1 to reverse. However unless acid is also added, it can readily be shown from available kinetic data that the rate of this reverse reaction is so slow that, for example, at the end of 2 hours in neutral solution the amount of free hydrogen peroxide present will have increased only about 10%.

The intensity of the color developed initially in the unmodified procedure corresponds to slightly more than the amounts of hydrogen peroxide plus hydroxymethyl hydroperoxide calculated to be present at equilibrium in the concentrated solution before dilution.

In the unmodified procedure the color intensity at the end of 5 hours was about 105 to 110% of that expected if all the peroxide present were in the form of the titanium complex with hydrogen peroxide. Presumably practically all of the peroxide present is then in the form of hydrogen peroxide or hydroxymethyl hydroperoxide and is complexed. In the case of acetaldehyde, the equilibrium data indicate that a far greater portion of the peroxide will be in the form of hydrogen peroxide or the monohydroxy hydroperoxide than is the case with formaldehyde. Therefore no significant interference is noted in the unmodified procedure in this case.

One must be careful to distinguish between an ordinary organic hydroperoxide, RCH_2OOH, and a hydroxyalkyl hydroperoxide, RCH(OH)OOH. The latter is a weak addition compound of an aldehyde and hydrogen peroxide which gives color formation with titanium. The former does not apparently complex with titanium to any appreciable extent as discussed previously.

The titanium sulfate reagent is reported to hydrolyze to form a precipitate at a pH over 0.8 (*3*). In the unmodified procedure where the acidity in the colorimeter cell is approximately 0.5*N*, it was observed that over the course of about 4 hours the absolute transmittance decreased by 5 to 10%, and a slight opalescence appeared. However the rate of development of turbidity was unaffected by the presence of formaldehyde or hydrogen peroxide, so the use of a blank, as was done in all tests reported here, eliminated errors from this source.

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Determination of Titanium in Titanium Metal

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The development of new titanium-base alloys, containing iron, chromium, molybdenum, tin, manganese, aluminum, magnesium, nickel, cobalt, copper, silicon, tungsten, and vanadium has led to a search for faster, accurate methods for the direct determination of titanium. The method developed uses the Eberbach Dyna-Cath, high speed magnetic mercury cathode to separate titanium from the most common and interfering elements associated in high-purity titanium metal and titanium alloys. Titanium is then determined colorimetrically as the yellow pertitanic acid. A significant saving in time has been accomplished with less care necessary to obtain the accuracy comparable to other methods.

TO MEET the need of the expanding titanium industry and new metallurgical advances, accurate and more rapid methods for the determination of impurities in titanium metal have been developed, especially since 1950. A method has been developed for the direct determination of titanium in high-purity titanium and titanium alloys, which combines speed with accuracy and without many painstaking conditions as in existing methods (*1*, *3*, *7*, *8*). While the theoretical bases for the method are not new, the adaptation of the procedure to titanium and titanium alloys compares favorably with other existing methods (*1*, *7*, *8*).

Methods for the determination of titanium in titanium metal have been reported. Knecht and Hibbert (*4*) first employed a method using a standard solution of ferric salt for the determination of titanium. It was modified later to include the use of thiocyanate added directly to the test solution instead of an outside indicator (*5*, *6*). Thompson (*8*) modified this method adapting it to the determination of titanium in high-purity titanium metal. The method offers satisfactory results and reproducibility, but it possesses certain disadvantages in that an approximate titration must be made before the actual determination in most cases, and the removal of impurities is tedious.

The method described uses the Eberbach Dyna-Cath (*2*), high speed magnetic mercury cathode, to separate titanium from chromium, molybdenum, iron, tin, cobalt, nickel, copper, and manganese, the most common and interfering elements associated with titanium. Small amounts of aluminum, silicon, magnesium, oxygen, tungsten, and vanadium do not interfere with the colorimetric determination of titanium as the yellow or orange pertitanic acid. For most alloys, the amount of tungsten and vanadium present is usually under 0.04% and no interference has been observed while working with relatively small samples.

APPARATUS AND REAGENTS

Apparatus. Dyna-Cath, Eberbach Corp., high speed magnetic mercury cathode.

Klett-Summerson photoelectric colorimeter. Beckman quartz spectrophotometer, Model DU. Volumetric flasks, 200-, 100-,

and 10-ml. capacity. Erlenmeyer flasks, 500-ml. capacity. Flask tongs. Beakers and covers.

Reagents. Potassium bisulfate, fused, pure.

Sulfuric acid (1 to 3), (1 to 5), and (1 to 9). Hydrogen peroxide, C.P., 3%.

National Bureau of Standards standard samples, Nos. 154 and 121b.

Standard titanium sulfate solution (1 ml. = 0.0001 gram of titanium).

Preparation. Fuse 0.34 gram of titanium dioxide (sample 154) in a platinum dish with approximately 10 grams of potassium acid sulfate and dissolve the cold melt in 200 ml. of hot sulfuric acid (1 to 9). Cool to room temperature and dilute to 2 liters in a volumetric flask with sulfuric acid (1 to 9).

PROCEDURE FOR DETERMINATION OF TITANIUM

Weigh accurately 0.20 gram of the sample and transfer to a 500-ml. Erlenmeyer flask. Add 100 ml. of sulfuric acid (1 to 5) and heat over a hot plate until completely dissolved. This operation requires approximately 15 minutes. Remove from the hot plate and cool. Metallic tungsten is insoluble in the nonoxidizing acid (*7, 9*) and minute black particles of metallic tungsten may separate in the solution. Since the amount of tungsten actually present in most titanium alloys is small, it may not be visible to the naked eye. If such a residue is present or suspected, remove by filtration on a No. 40 Whatman filter paper. Vanadium (*1, 7, 9*), also in minute quantity if present, for the most part accompanies the tungsten and is removed with it.

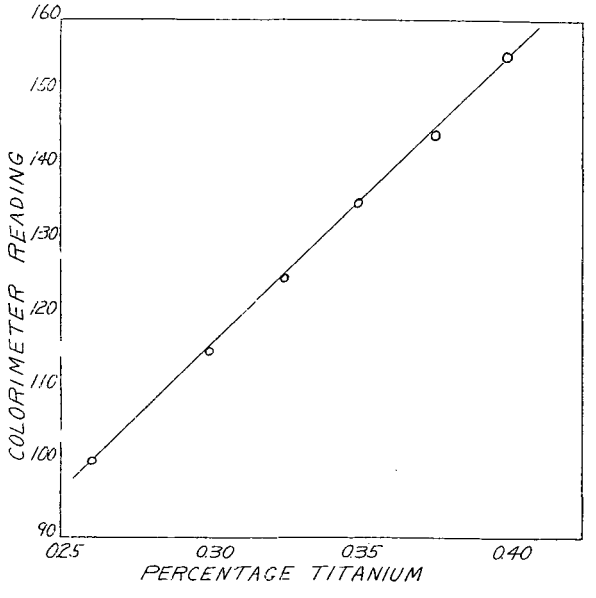

Figure 1. Titanium in titanium-base alloys

Add 50 ml. of 3% hydrogen peroxide to the filtrate; the solution changes from a greenish blue to a yellow orange. Evaporate the solution until the yellow color disappears, then to fumes of sulfur trioxide. Do not fume strongly. Cool, add 75 ml. of distilled water, and boil for 5 minutes. Cool, and place the solution, washing well the sides of the flask, in the Dyna-Cath cell beaker, containing approximately 1.5 pounds of clean mercury. Cover the cell beaker and electrolyze the electrolyte for approximately 15 minutes at 10 to 15 amperes, to remove chromium, molybdenum, tin, cobalt, nickel, copper, zinc, iron, and most of the manganese from the electrolyte. For total alloy contents of less than 10%, the solution may assume the yellow color of pertitanic acid. Interrupt the electrolysis when this occurs and remove the electrolyte from the cell, washing well the inside of the cell and cathode with small quantities of distilled water. The electrolyte may be clear or yellowish in color. If the volume of the solution is more than 200 ml., place the contents on the hot plate in a 500-ml. beaker and evaporate to approximate volume. Cool, make up to volume in a 200-ml. volumetric flask, and pipet 2 ml. of the homogeneous solution in a 100-ml. volumetric flask. Add 20 ml. of sulfuric acid (1 to 3) and 50 ml. of distilled water. Add 5 ml. of 3% hydrogen peroxide and make up to volume. Compare the color reading against known values of the standard titanium sulfate solution or compare reading, using blue filter No. 42 on the Klett-Summerson, to the calibration curve, Figure 1, determined by using Bureau of Standards standard samples 121b and 154. Percentage reading × 250 = % titanium in sample, when a 0.20-gram sample is taken.

Table I. Determination of Titanium in Titanium Metal

Nominal Compn. of Alloy, %	Titanium Determined, % Thompson's method (*8*)	Klett-Summerson	Beckman
Fe 0.50, N 0.03, C 0.04, O 0.50, Mn 0.05, W 0.03	99.00	99.04	99.06
	99.02	99.06	99.06
	98.89	99.01	99.03
	98.97	99.01	99.04
Ti-150A	95.89	95.89	95.94
Fe 1.30, Cr 2.50, O 0.30, C 0.02, N 0.025, W 0.04	95.92	95.96	95.94
	95.86	95.89	95.90
	95.86	95.91	95.92
	95.84	95.89	95.92
	95.86	95.87	95.89
Ti-175A	94.03	94.01	94.04
Fe 1.75, Cr 3.00, O 0.50, N & Mn 0.04, C 0.03, W 0.02	94.02	94.05	94.06
	93.96	93.98	94.02
	93.98	93.98	94.02
Fe 2.00, Cr 2.00, Mo 2.00, C 0.04, O 0.40, N & W 0.02	93.67	93.76	93.78
	93.70	93.74	93.75
	93.66	93.72	93.74
Fe 2.00, Cr 2.00, Mo 2.00, C 0.04, O 0.40, N & W 0.02	92.80	92.80	92.82
	92.76	92.81	92.80
	92.72	92.75	92.80
RC-130B	92.08	92.31	92.38
Mn 4.00, Al 4.00, O 0.30, N 0.05, C 0.06	92.12	92.31	92.31
	92.18	92.30	92.28
	92.18	92.26	92.28
	92.20	92.30	92.30
Ti-150B	85.80	85.87	85.90
Cr 5.00, Mo 5.00, Fe 5.00, O 0.30, N 0.03, C 0.04	85.84	85.87	85.92
	85.78	85.88	85.90

A similar curve for the Beckman spectrophotometer may be determined using the same standards and measuring the percentage of absorbance or transmittance at 425 mμ on the Beckman. The results appear in Table I.

DISCUSSION

The results obtained were found to be equally as accurate as those obtained by Thompson. The results obtained on the Beckman spectrophotometer can be considered more precise than the results obtained on the Klett-Summerson. The time involved in the two methods was in favor of the direct determination with an average time of 80 minutes. Less care is necessary to obtain the same accuracy.

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Nonaqueous Titration of Dilute Acids and Bases in Acrylonitrile

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Precise methods developed for the determination of small amounts of weak and strong acids and bases in acrylonitrile are rapid and free of interference from atmospheric carbon dioxide. The application of nonaqueous techniques has made possible the titration of low (0.002 weight %) concentrations of choline hydroxide, as well as acetic and acrylic acids with a precision of ±0.001 weight %. The procedures do not require blank corrections or special equipment, and thus results are more rapidly obtained and are more reliable than those obtained by the usual aqueous procedures.

CERTAIN investigations made it necessary to develop rapid, reproducible methods for the routine determination of small amounts of organic acids and bases in acrylonitrile. For example, solutions of acrylonitrile containing 0.0005 to 0.0050 weight % of the strong organic base, choline [(2-hydroxyethyl)-trimethylammonium hydroxide], were required to be analyzed with a high accuracy and precision. Acrylonitrile solutions containing similar concentrations of acrylic and acetic acids were also under study.

The colorimetric procedure of Dyer (*1*) is applicable for choline and possibly choline salt, but the intensity of the color was found to be sensitive to small amounts of water. Acrylonitrile contains small, but varying, amounts of water which precluded the use of this method.

The determination of acids in organic solvents and monomers is usually carried out by extraction or dissolution (*2*) with water followed by titration of the extract employing standard aqueous acidimetric procedures. For precise determinations the water must be carbon dioxide–free and the titration must be carried out in a carbon dioxide–free atmosphere. Alternatively, blanks may be run to correct for carbon dioxide interference. When the values expected are 0.02% acid or higher these procedures usually suffice. However, some of the mixtures in this study contained less than 0.002% acetic or acrylic acids and preliminary experiments indicated that the blank corrections frequently were several times larger than the known acid concentrations.

A more direct approach to both problems, acids and bases, seemed available in the recently emphasized methods of nonaqueous titration. Choline, as a strong base, and even the more weakly basic choline salts seemed amenable to titration, provided suitable titrants, solvents, and indicators could be discovered. Shortly after the laboratory phase of this work was completed, the publication by Markunas and Riddick (*4*) of nonaqueous determination of choline salts of carboxylic acids pointed up the effectiveness of this approach. Their work involved the assay of certain pure choline salts; however, the present discussion is concerned with trace analyses. Another recent reference of interest is that of Keen and Fritz (*3*), who describe the nonaqueous titration of small amounts of amines.

The literature abounds in applications of nonaqueous techniques to assay problems and macrotitrations, but the nonaqueous determination of trace components in high-purity commercial products seems to be a fruitful field, and these approaches are expected to become increasingly important. Indeed, the methods described here are recommended for consideration in routine application to the determination of traces of acids or bases in the specification analyses of commercial acrylonitrile samples. Application to other monomers and solvents should be obvious.

METHODS

The procedures used in this paper apply both potentiometric and indicator methods of detecting the end points. Seaman and Allen (*7*) suggested application of a potentiometric determination of the end point for each new acid or base considered. In this manner it is possible to screen indicators and to determine the precise indicator shade at which an equivalence point has been reached. Bromothymol blue gives a sharp color change from yellow to blue when titrating as little as 0.001 weight % of acrylic or acetic acid in acrylonitrile. In titrating choline with standardized acid the reverse color change, from blue to yellow, occurs. The transition through a green shade is rapid.

Although in routine practice, the indicator titrations are carried out directly in acrylonitrile without the addition of a solvent, potentiometric titrations involving acrylonitrile require the addition of solvents, especially, when commercially available glass and calomel electrodes are used. The G-H (glycol-hydrocarbon) solvent system described by Palit (*5*) proved satisfactory for the titrations of acids and the strong bases, such as choline or benzyltrimethylammonium hydroxide. For weaker bases, such as choline salts, the acetic acid solvent of Pifer and Wollish (*6*) gave good potentiometric end points. In this latter system, the visual indicator found most useful was crystal violet.

REAGENTS AND APPARATUS

Indicator Solutions. Dissolve 100 mg. of Harleco bromothymol blue in 100 ml. of C.P. methanol and adjust to the neutral (green) with dilute (0.01*N*) sodium hydroxide. Crystal violet indicator is made up to be 0.1% in acetic acid.

Standardized Perchloric Acid Titrant, 0.02*N*. Dissolve 1.70 ml. of 72% perchloric acid (G. Frederick Smith Chemical Co.) in 1 liter of C.P. dioxane. Standardize potentiometrically against potassium acid phthalate dissolved in C.P. dioxane.

Standardized Methanolic Sodium Hydroxide, 0.02*N*. Dissolve 0.80 gram of C.P. sodium hydroxide in 1 liter of C.P. methanol. Protect from carbon dioxide absorption with an Ascarite tube on the buret.

Glycol-Hydrocarbon Solvent. Equal volumes of ethylene glycol (C.P.) and isopropyl alcohol (C.P.) are mixed. If technical grade solvents are used, corrections for traces of acids must be determined.

pH Meter. Beckman Model H2, with standard glass and calomel electrodes, with magnetic stirrer was used.

PROCEDURE

Organic Acids. To a 25-ml. sample of acrylonitrile containing organic acids add 6 drops of alcoholic bromothymol blue indicator. Titrate with 0.02*N* methanolic sodium hydroxide using a microburet until the color changes from yellow to blue. The color change is sharp.

Organic Bases. To a 25-ml. sample of acrylonitrile containing choline or other strong base add 6 drops of bromothymol blue indicator. Titrate to the green end point using standardized (0.02*N*) perchloric acid in dioxane.

Weak Organic Bases (basic salts of choline). To a 25-ml. sample of acrylonitrile containing organic bases add 25 ml. of glacial acetic acid and 4 drops of crystal violet. Titrate with 0.02*N* perchloric acid in dioxane or acetic acid using crystal violet indicator. The indicator changes from blue to green at the equivalence point.

Potentiometric Titrations. In the determination of acids and strong bases 50 ml. of glycol-hydrogen solvent is added to a beaker and the apparent pH recorded. Acrylonitrile (25 ml.) is then added, with stirring. The deflection in apparent pH is observed and acid or base titrant is added as required until

Table I. Nonaqueous versus Aqueous Methods

	Known Wt. %	Found Wt. % Nonaqueous[a]	Found Wt. % Aqueous[b]
Acetic acid in purified acrylonitrile	0.0020	0.0020	0.0060
	0.0035	0.0040	0.0070
Commercial acrylonitrile		0.0030	0.0110
			0.0120
			0.0140

[a] Indicator titration, bromothymol blue.
[b] Indicator titration, phenolphthalein.

Table II. Indicator[a] Titrations

	Weight % Known	Found	Abs. error
Acetic acid in acrylonitrile	0.0020	0.0020	0.0000
	0.0032	0.0041	0.0009
	0.0071	0.0069	0.0002
	0.0120	0.0114	0.0006
Acrylic acid in acrylonitrile	0.0013	0.0015	0.0002
	0.0028	0.0035	0.0007
	0.0066	0.0062	0.0004
	0.0085	0.0080	0.0005
Choline in acrylonitrile	0.0005	0.0003	0.0002
	0.0008	0.0008	0.0000
	0.0010	0.0011	0.0001
	0.0020	0.0016	0.0004
	0.0040	0.0031	0.0009
	0.0080	0.0072	0.0008
Basic choline salt in acrylonitrile	0.0047	0.0040	0.0007
		0.0036	0.0011
	0.0089	0.0083	0.0006
		0.0083	0.0006
	0.0095	0.0078	0.0017
	0.0132	0.0119	0.0013
	0.0205	0.0203	0.0002

[a] Bromothymol blue indicator.

Table III. Statistical Data

	Range Found, Wt. % Acetic Acid	No. of Detn.	Std. Dev.[a], Wt. %
Commercial acrylonitrile	0.0004–0.0009[b]	8	0.00014
Acrylonitrile plus 0.0044% of acetic acid	0.0047–0.0050[b]	3	0.00015
Acrylonitrile plus 0.0088% acetic acid	0.0094–0.0099[b]	7	0.00020
	0.0079–0.0083[c]	7	0.00019

[a] Standard deviation at 1-σ level.
[b] Indicator (bromothymol blue) titrations.
[c] Potentiometer (G-H solvent) titrations.

the pH is returned to the original value. The titrant equivalent required for this operation is then calculated.

In the titration of weaker bases than choline, such as carboxylic acid salts of choline, the preferred potentiometric solvent is C.P. acetic acid with standard perchloric acid in acetic acid or dioxane as the titrant. For weak bases, plot the readings from the electromotive force scale, rather than pH, against titrant volume and determine the end point as the increment of titrant causing the largest potential change.

DATA AND DISCUSSION

The usual aqueous methods of titrimetry were applied to the first samples studied. This was done by diluting acrylonitrile with 15 volumes of distilled water, followed by blank corrections for carbon dioxide present in the water. Although carbon dioxide–free water was used, and care was taken to minimize carbon dioxide absorption during the titrations, the blanks were high compared to the acid and base concentrations expected. The simpler nonaqueous procedures gave more reasonable values when applied to purified (redistilled) acrylonitrile to which small amounts of choline or acrylic acid had been added. Table I indicates the recoveries of acetic acid by both aqueous and nonaqueous methods. The procedures described gave very satisfactory recoveries.

Table II relates experimentally determined values to calculated amounts of acetic and acrylic acids and choline as well as choline salts. The variations found were generally less than 0.001 weight % and exhibited no constant bias in the cases of acids and strong bases. However, in the case of weak bases, the results were frequently low, occasionally by as much as 0.0015 weight %, which was within the precision required for routine determinations during this study.

The precision, defined as the standard deviation at the 1-σ level, of several samples is shown in Table III. These show the precision to be 0.0002 weight % for this series of samples.

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Circular Paper Chromatographic Method for Estimation of Thiamine and Riboflavin in Multivitamin Preparations

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A simple circular paper chromatographic method has been developed for the separation and simultaneous estimation of vitamins B_1 and B_2 in multivitamin preparations.

THE separation, identification, and quantitative estimation of B-complex vitamins by paper chromatography has been drawing the attention of many workers in recent years. Beran and Sicho (*2*) reported the conversion of thiamine into thiochrome on paper by treatment with alkaline ferricyanide and detected it by its blue fluorescence under ultraviolet light. Miyaki and others (*12*) described a procedure by which the thiamine in 95% ethyl alcohol containing 10% sodium hydroxide was chromatographed and identified by spraying with diazotized *p*-aminoacetophenone and alkali, and applied this procedure for the quantitative estimation of thiamine by an area method. Fried (*5*) suggested a paper electrophoretic separation of thiamine from other fluorescent substances. A paper partition chromatographic method was described by Crammer (*4*) for the separation and identification of riboflavin and other flavine compounds. Hais and Pecakova (*9*) used this method for following the photolysis products of riboflavin and applied it for the analysis of commercial injection solutions.

A method for the paper chromatographic separation and determination of some water soluble vitamins suitable for the analysis of multivitamin preparations was reported by Brown and Marsh (*3*), who measured the areas of discrete zones of total ultraviolet absorption with the help of a Cary spectrophotometer. Radhakrishnamurty and Sarma (*14*) reported the separation of various members of the B-complex vitamins by ascending paper chromatography and identified the individual vitamins on the paper by specific spot tests. Recently, Giri (*7*) described briefly a simple method for the estimation of thiamine and riboflavin by circular paper chromatography.

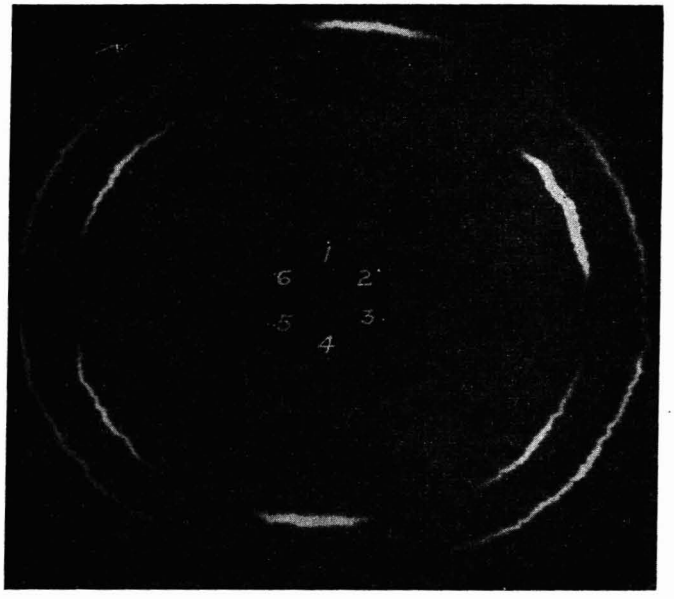

Figure 1. Circular paper chromatogram showing fluorescent bands of thiochrome and riboflavin

Photographed under Philips HPW 125 W analyzing lamp with saturated sodium nitrite solution as filter

1. Standard thiamine hydrochloride
2. Becozym (Roche)
3. Becozym Forte (Roche)
4. Standard riboflavin
5. Vitaminets (Roche)
6. Becadex (Glaxo)

The thiochrome method (*13*) generally used for the estimation of thiamine in pharmaceutical preparations is time-consuming and cumbersome. A recent method reported by Bandelin and Tuschoff (*1*) for the colorimetric determination of thiamine in pharmaceutical products claims to overcome certain of the disadvantages of the fluorescence method. A simple paper chromatographic method for the separation and simultaneous estimation of thiamine and riboflavin in multivitamin tablets and capsules is described in this paper.

APPARATUS AND REAGENTS

Filter Paper. Whatman No. 1, 18 or 24 cm. in diameter.

Developing Solvent. The solvent used was 1-butanol–acetic acid–water (4 to 1 to 5). The solvent was prepared by shaking 4 parts of 1-butanol with 1 part of glacial acetic acid and 5 parts of water and rejecting the lower layer on separation, the upper layer being used as the solvent.

Vitamin Solutions. A 0.02% solution of pure thiamine hydrochloride (Hoffman-La Roche) was prepared by dissolving 20 mg. of vitamin in 20% alcohol and diluting it to 100 ml. with water. A 0.01% solution of riboflavin was prepared by dissolving 10 mg. of the pure vitamin in water by the addition of a small amount of glacial acetic acid and warming on a water bath and diluting the volume to 100 ml.

Solutions of Multivitamin Preparations. Multivitamin Tablets. The tablets were powdered in a mortar, and the powder was first digested with 30 ml. of water containing a few drops of glacial acetic acid (to facilitate the dissolution of riboflavin) by heating on a water bath for 0.5 hour. The extract was allowed to settle, and the supernatant fluid was decanted into another flask. The residue was once again digested on the water bath with a similar amount of water containing glacial acetic acid. The combined extracts were filtered, the residue was washed well with water, and the filtrate was diluted to 100 ml.

Multivitamin Capsules. As the soft gelatin capsules like Enervit (Mac Laboratory) and Vimagna (Lederle Laboratories) contain the oil-soluble vitamins also, the capsules were pierced with a pin and macerated in a mortar with several 20-ml. portions of ether until all the contained material was extracted. The ether extract was transferred to a separatory funnel, and the water-soluble vitamins were extracted with water containing a few drops of glacial acetic acid. The combined extracts were diluted to suitable volumes.

Cyanogen Bromide Solution. Cyanogen bromide solution was prepared fresh every time by the addition of 10% potassium cyanide solution drop by drop to saturated bromine water until the latter was just decolorized. The preparation of the reagent was carried out in a fume cupboard.

Micropipets. Pipets calibrated for 11.2 μl. were used to apply the vitamin solutions to the paper.

Petri Dishes. Petri dishes 4 and 6 cm. in diameter were used as the inner and outer containers for the conversion of thiamine to thiochrome.

Ultraviolet Lamp. Philips H.P. 125W analyzing lamp was used.

Klett fluorimeter.

EXPERIMENTAL

The thiamine on the paper was converted to thiochrome by cyanogen bromide and alkali as described by Fujiwara and Matsui (*6*), who reported that this method is better than the alkali ferricyanide method of Janssen (*10*), as the amount of cyanogen bromide that can be used has a relatively broad range and the conversion of thiamine to thiochrome takes place over a fairly wide range of pH and is not affected by any reducing compound. The technique of circular paper chromatography described by Giri and Rao (*8*) was employed.

Thiamine and riboflavin solutions were spotted by means of the calibrated micropipet on the circumference of a circle (2.2 cm. diameter) drawn at the center of an 18-cm. Whatman No. 1 filter circle. While the spots were still damp, the filter circle was exposed for about 20 minutes to the vapors of cyanogen bromide and ammonia contained in two Petri dishes (one kept inside the other). The larger Petri dish contained ammonia and the smaller one contained cyanogen bromide solution. The whole setup was covered by a bell jar, and the operation was conducted in a well ventilated fume cupboard. When the filter paper was dry it was removed, and a cylindrical paper wick was placed at its center. It was then developed using the solvent (1-butanol–acetic acid–water). When the solvent boundary had run almost to the edge of the circle, the chromatogram was removed, air-dried, and observed under the filtered ultraviolet lamp. The fluorescent band of thiochrome and riboflavin could be seen distinctly on the chromatogram, the blue fluorescent band of thiochrome occupying the position above the green one of riboflavin (Figure 1). The fluorescent bands were marked with a pencil. Each band was then cut and eluted with 6 ml. of water and shaken in extraction cylinders. The fluorescence of the solutions was measured in a Klett fluorimeter using the necessary filters for vitamins B_1 and B_2. The fluorescence of water blanks was also measured and the thiamine and riboflavin contents of the preparations were determined.

RESULTS

Standard Curves for Thiamine and Riboflavin. Standard solutions of thiamine containing 0.56 to 4.48 γ were spotted on a 18-cm. filter paper by means of a calibrated micropipet. After conversion to thiochrome, the chromatograms were developed with the solvent, the bands were cut and eluted with water, and estimated in the fluorimeter. Riboflavin solutions of the same range were spotted on a different paper and developed with the same solvent. The standard curves for both the vitamins representing an average of four determinations are presented in Figure 2.

Analysis of Vitamin Mixtures. Vitamin mixtures were prepared at two concentrations—one containing 1.12 γ per 11.2 μl. each of vitamins B_1 and B_2 and the other containing 2.24 γ of each of these vitamins. The solutions were spotted and chromatogramed; and replicate determinations of vitamins B_1 and B_2 separated on the paper were carried out. The results are presented in Table I.

Analysis of Multivitamin Preparations. Suitable volumes (11.2 to 31.5 μl.) of the tablet solutions were spotted in duplicate on the circumference of a circle, 3 cm. in diameter, at the center of a 24-cm. circle. Standard solutions of vitamins B_1 and B_2 containing 1.12 γ of each were also spotted in duplicate on the same paper. For each tablet two such chromatograms were prepared, and the average of the four readings was taken for the calculation of vitamin contents. The vitamin potency of the preparations has also been determined by other standard methods (*11*, *13*) and the values obtained are given for comparison in Table II.

Stability of Thiochrome and Riboflavin on Paper Chromatograms. Five aliquots of solutions of vitamin B_1 containing 1.12 γ were spotted on two different 18-cm. diameter filter papers. After conversion into thiochrome, the chromatograms were developed with the solvent and air dried. One band from each chromatogram was cut and eluted with water and estimated immediately. The second, third, fourth, and fifth bands were estimated after two, four, seven, and 14 days, respectively. The same experiment was repeated with 2.24 γ of thiamine. Another set of chromatograms was prepared for 1.12 and 2.24 γ of riboflavin, and the bands were estimated at the same intervals. The readings of the bands along with time intervals are given in Table III.

DISCUSSION

The standard curves show that from 0.56 to 2.24 γ the readings for vitamins B_1 and B_2 are linear, and hence quantitative estimations of these vitamins can be carried out with fair amount of accuracy in that range. Replicate determinations of vitamins B_1 and B_2 from mixtures and the analysis of multivitamin tablets show that the reproducibility of the method is about 10%. The thiochrome band appears to be stable on the paper, as indicated by the figures presented in Table III. The riboflavin also is stable for nearly a fortnight.

This method is simple, elegant, and less time-consuming and requires very small quantities of the solutions. The method can be safely used for the rapid, simultaneous determination of vitamins B_1 and B_2 in multivitamin preparations. A rough comparison of the fluorescence of the solutions could be made even by visual observation.

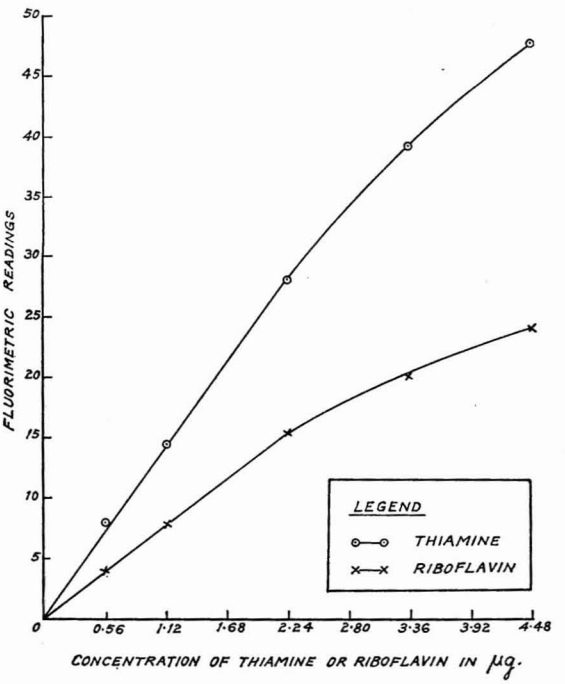

Figure 2. Calibration curves for thiamine and riboflavin

Table I. Analysis of Vitamins B_1 and B_2 in Mixtures

Concentration I[a]		Concentration II[b]	
Thiamine, γ	Riboflavin, γ	Thiamine, γ	Riboflavin, γ
1.6	1.06	2.10	2.12
1.05	1.10	2.14	2.12
1.08	1.05	2.12	2.09
1.09	1.14	2.09	2.07
1.09	1.12	2.12	2.06
1.06	1.10	2.06	2.16
1.10	1.04	2.04	2.16
1.07	1.06	2.18	2.20

[a] 1.12 γ of each vitamin.
[b] 2.24 γ of each vitamin.

Table II. Analysis of Vitamins B_1 and B_2 in Multivitamin Preparations

Preparation	Thiamine, Mg.		Riboflavin, Mg.	
	Thiochrome method	Chromatographic method	Fluorescence method	Chromatographic method
Becadex	3.25	2.95	2.05	1.95
C.C.F. vitamin B complex	10.65	9.95	3.20	2.95
Vimagna	3.60	3.15	3.20	2.85
Inervit	1.42	1.48	1.95	2.15
Becozym	5.65	4.95	1.90	2.10
Becozym Forte	15.95	15.15	16.40	15.25
Vitaminets	4.55	4.05	2.25	1.95

Table III. Stability of Thiochrome and Riboflavin on Paper Chromatograms

No. of Days	Fluorimeter Reading, γ			
	Thiamine		Riboflavin	
	1.12	2.24	1.12	2.24
0	12	22	8	15
2	14	23	7	12
4	12	25	6	13
7	15	24	6	12
14	14	23	6	11

ACKNOWLEDGMENT

The authors are indebted to Volkart Brothers for their gift of the samples of multivitamin tablets.

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Barite Analysis with X-Ray Spectrograph

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A procedure has been developed using the x-ray spectrograph, in which barium L beta$_1$ radiation is used for the quantitative analysis of barite ores. A working curve was established by a series of standard samples over the range 25.6 to 100% of barium sulfate. X-ray spectrographic results are in agreement with wet chemical analysis to within 0.5% barium sulfate. The total time for analysis, after the sample has been ground, is approximately 3 minutes.

THE determination of barium in samples containing barite may be rather time-consuming by ordinary wet chemical analysis. By utilizing the superior speed of the x-ray spectrograph, barium analyses may be carried out on barite samples in approximately 3 minutes, once a working curve has been established.

APPARATUS

A North American Philips x-ray spectrograph with a tungsten x-ray tube and sodium chloride analyzing crystal was used throughout the analytical work. The following operating conditions were used:

Target	Tungsten
Analyzing crystal	Sodium chloride
X-ray tube operated at	50 Kv., 40 ma.
Operation of scaler	Fixed count
Scale factor	32
Geiger tube operated at	1500 volts
Analysis line	Ba Lβ_1 54.16° (2θ)

The spectrometer was aligned according to standard procedure with pure nickel foil (*1*).

METHOD

A series of barite samples, obtained from the Research Foundation at the Colorado School of Mines, which had been previously chemically analyzed for their barium content, were used in constructing the working curve. The matrix of these standard samples consisted primarily of silica, magnesium silicate, and monocalcium aluminum silicate. The samples were thoroughly dried and ground to −400 mesh in a disk-type pulverizer. The amount of iron contamination in grinding to this particle size range, determined by qualitative spectrographic analysis, was less than 0.1%. All samples were ground in the same manner, so that contamination was essentially constant throughout all samples.

In order to reduce the probable statistical error in counting, four readings on each sample were taken consecutively without resetting the interval timer or the scaler circuits. Since the statistical error in counting is primarily dependent on the total count, similar results should be obtained by setting the scale factor to 128 and counting once. Any possible cascading of count may be averaged out to a greater degree by four separate counts, however.

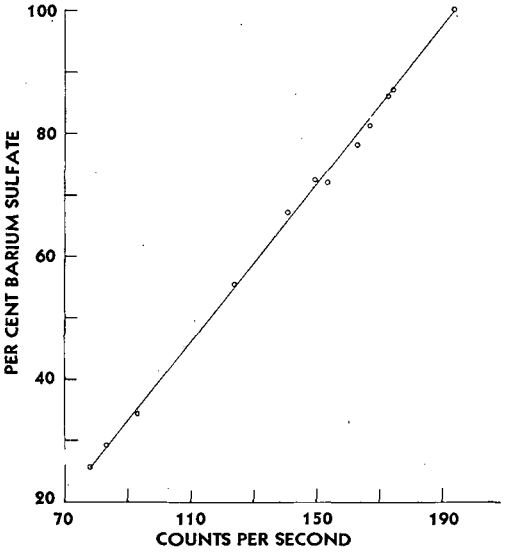

Figure 1. Working curve for barium sulfate

Table I. Counting Rate for Standard Barium Sulfate Samples

Barium Sulfate, %	Counts per Second			
	1st day	2nd day	3rd day	Average
25.6	79.1	77.9	77.3	78.1
29.1	83.4	84.0	81.2	82.9
34.9	96.0	93.4	91.7	93.7
55.8	123.1	123.4	122.6	123.0
67.3	142.5	141.7	140.2	141.5
72.4	152.2	154.4	152.3	153.0
72.7	149.9	148.8	146.7	148.5
78.0	164.7	161.8	163.0	163.2
81.2	168.9	167.1	167.2	166.1
86.4	171.3	172.5	174.4	172.7
86.9	172.3	174.9	172.8	173.3
100.0[a]	195.1	191.1	194.2	193.5

[a] C.P. barium sulfate.

Table II. Maximum Deviation of Barium Sulfate Concentrations from Average

Barium Sulfate, %	Av. of 3 Runs, Counts/Sec.	Max. Dev. from Av. % Barium Sulfate
25.6	78.1	0.33
29.1	82.9	0.59
34.9	93.7	0.86
55.8	123.0	0.18
67.3	141.5	0.61
72.4	153.0	0.66
72.7	148.5	0.68
78.0	163.2	0.71
81.2	166.1	1.37
86.4	172.7	0.70
86.9	173.3	0.80
100.0	193.5	0.83
	Av. dev.	0.69

WORKING CURVE

A series of samples ranging in barium sulfate content from 25.6 to 100% were used to construct the working curve. Counting rate readings were taken over a 3-day period, and the results averaged to obtain the final working curve.

No background correction was found necessary in the construction of the working curve over the concentration range involved in this series of barite samples. The absorption and scattering of x-rays by the matrix material, silicon, aluminum, and calcium, all low atomic-numbered elements, were essentially constant, although the relative amounts of matrix material were varied. The presence of excessive amounts (greater than 20%) of higher atomic-numbered elements in the matrix would be expected to alter the slope of the working curve and corrections to the curve as given in this paper would have to be made (*2*).

The results of this series of data are given in Table I and Figure 1.

A check of the maximum statistical error involved in counting was made using the data as recorded from the series of standard samples given in Table I. The results of this work are given in Table II.

By using a scale factor of 256 or a total count of 4 × 25,600 counts, the average deviation should be reduced to approximately 0.23% barium sulfate. The total counting time would, however, be approximately doubled for each sample.

ANALYSIS OF BARIUM SULFATE SAMPLES

Four samples for which chemical analyses were known were selected at random for comparison of chemical and x-ray spectrographic analysis, using the working curve established in Table I and Figure 1. The results of this analysis are given in Table III.

Table III. Comparison of Chemical and X-Ray Spectrographic Analysis for Barium Sulfate

	% Barium Sulfate	
Counts/Sec.	X-ray spectrograph	Wet chemical analysis
142.4	66.7	66.52
177.6	89.5	89.12
185.3	94.6	94.12
190.4	98.1	98.32

CONCLUSIONS

The working curve for barium sulfate established on the x-ray spectrograph follows a straight line in the upper concentration ranges, and the results are reproducible to nearly within statistical fluctuations in counting. Results based on the spectrographic method agree with wet chemical analysis to within 0.2 to 0.5% barium sulfate. Although no standard samples were available in the very low concentration ranges for barium sulfate, the working curve should be able to be extended to low concentrations of barium sulfate, perhaps to values of a few tenths of 1% barium sulfate by changing the scale factor. The method should be useful for routine control analysis on large numbers of samples, because the time for an analysis is very short. For example, the total counting time for the 72.7% barium sulfate samples was 4 × 3200 ÷ 148.5 seconds or 1 minute 16 seconds.

Samples must be thoroughly dried before using, and preferably ground to −400 mesh, as the precision of the x-ray spectrograph decreases with larger particle sizes. Sample handling is at a minimum, the only handling required being to place the sample (approximately 30 grams) in the plastic sample holder. By keeping the surface of the sample at the same level, and using the same surface area, smaller size samples (less than 30 grams) could also be counted.

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Determination of Organic Substances by Standard Chromous Chloride Solution

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A standard chromous chloride solution of exactly determinate strength was utilized for the determination of a number of reducible organic compounds. These include anthraquinones, nitro, nitroso, azo, and acetylenic compounds. The anthraquinones were titrated directly with chromous chloride, while the other compounds were analyzed by adding an excess of reducing agent and back-titrating with standard ferric alum solution. The end points were determined potentiometrically.

CHROMOUS salts have not been as extensively used for the quantitative analysis of reducible organic compounds as titanous salts. Someya (*4*) reduced *p*-nitroaniline, picric acid, and *p*-nitrophenol with an excess of chromous chloride solution which was prepared by the incomplete reduction of chromic chloride by amalgamated zinc. The excess chromous chloride was titrated with standard ferric alum solution. Terent'ev and Goryacheva (*5*) had titrated quinone, azobenzene, and *m*- and *p*-nitroaniline directly using methyl red as an indicator. Their precision for the determination of azobenzene was very poor. They prepared their chromous solution by dissolving chromous acetate in hydrochloric acid. Both of these methods necessitated the frequent standardization of the chromous solution. Recently, Lingane and Pecsok (*1*) have shown that it is relatively easy to prepare and maintain a standard chromous solution of exactly determinate strength. Since chromous solutions are stronger reducing agents than titanous solutions, and their reactions are generally faster than titanous salts in that the reductions are usually carried out at room temperature, it was decided to reinvestigate the use of chromous chloride for organic analysis.

This paper presents the results of the use of this reagent for the determination of: *o*-nitrobenzoic acid, 2,4,6-trinitrobenzoic acid, 2,4,6-trinitroresorcinol, 2,4-dinitrophenylhydrazine, nitroguanidine, *p*-nitrobenzeneazoresorcinol, nitroso R salt, anthraquinone 2,7-disodium sulfonate, and the monopotassium salt of acetylene dicarboxylic acid. All are quantitatively reduced, the nitrogen-containing compounds to the corresponding amines (rupture of the *N*—*N* link in the azo compound), the anthraquinone to the corresponding anthrahydroquinone, and the acetylenic compound to the corresponding ethylenic compound.

EXPERIMENTAL

Apparatus. The titration cell was a tall-form 200-ml. electrolytic beaker, covered by a rubber stopper provided with a gas inlet tube, a saturated calomel reference electrode, a platinum indicator electrode, a thermometer, if the reaction was to be carried out at an elevated temperature, and openings for a gas outlet and the delivery tips of two burets. If any of the openings were not used, they were closed by means of corks. If the solution was to be heated, a beaker encircled with asbestos-covered heating wire was used. The temperature was controlled by regulating the current flowing through the heating wire by means of a Variac.

The solutions were stirred with a magnetic stirrer.

The end point of the reaction was determined potentiometrically by measuring the voltage change by means of a Leeds and Northrup line-operated pH meter, Model 7664. Since the titrant could be added in very small increments (about 0.02 ml., if one

spins the stopcock rapidly and the buret tip has a rather small opening), it was not necessary to plot the voltage change. The voltage change at the end point in the back-titration of excess chromous chloride with ferric alum solution is about 500 mv., while the voltage change at the end point in the direct titration of the anthraquinone salts is only about 175 mv.

The end point in the back-titration of chromous solution with ferric alum solution can also be determined using the derivative polarographic end point (*3*), in which case the saturated calomel reference electrode is replaced by a second platinum electrode. The pair of platinum electrodes are polarized by a small constant current of about 2 μa.

The apparatus used for storing and dispensing standard chromous solution was the same as that utilized by Lingane and Pecsok (*1*), except that a 2-liter storage flask was used in place of the 1-liter one. In this way enough chromous solution was available for about 35 separate determinations.

Reagents. A standard 0.1000*N* solution of chromous chloride in 0.1*N* hydrochloric acid was prepared directly in the storage flask by the procedure described by Lingane and Pecsok (*1*). The 2-liter storage flask was filled about two thirds full with amalgamated mossy zinc, and about 1 liter of 0.1000*M* chromic chloride in 0.1*N* hydrochloric acid was added. The reduction was usually allowed to proceed overnight. The solution was stored under pure hydrogen obtained from a Kipp generator. The hydrogen was freed from oxygen by passage through a bubble tower containing chromous chloride solution in 1*N* sulfuric acid in contact with amalgamated zinc. The chromous solution was standardized against standard cupric solution in 6*N* hydrochloric acid as recommended by Lingane and Pecsok (*1*). The standard cupric solution was prepared from copper sulfate pentahydrate and was standardized electrogravimetrically as described by Willard and Furman (*7*).

Baker and Adamson's "reagent quality" zinc was amalgamated with about 2% of mercury by shaking it for about 10 minutes in a mercuric chloride solution in dilute hydrochloric acid. At first, a solution of mercuric nitrate in dilute nitric acid was used to amalgamate the zinc; however, the chromous solution obtained by using this amalgamated zinc always had a normality about 2 to 3% too low, although the amalgam was washed thoroughly before use.

An approximately 0.1*N* ferric alum solution, acidified with sulfuric acid (to 1*N*), was used in the back-titration of excess chromous chloride solution. The solution was freed of oxygen by passing nitrogen through it for about 15 minutes. The nitrogen was freed of oxygen in the usual manner. The solution was standardized either by titrating with standard permanganate (*6*) portions of the solution which were reduced by amalgamated zinc in a Jones reductor or by titrating aliquots with standard chromous solution.

Standard solutions of the organic compounds investigated were generally prepared by dissolving a known weight of the purest commercially available material in water, or in glacial acetic acid, if the substance was insoluble in water. The sample of nitroguanidine was recrystallized from water two times and air dried. One of the solutions of the monopotassium salt of acetylene dicarboxylic acid was prepared from material synthesized according to the method of Moureu and Bongrand (*2*).

Procedure. Except where otherwise specified the following procedure was employed. Suitable aliquots of the solution to be analyzed were pipetted into the titration vessel which contained about 15 ml. of water. Ten milliliters of concentrated hydrochloric acid were added, and the initial volume of the solution was adjusted to about 50 ml. by adding water. Carbon dioxide, freed from traces of oxygen by passage through acidified chromous solution in contact with amalgamated zinc, was bubbled through the solution for about 10 minutes. At the end of this period the carbon dioxide was passed over the surface of the solution. An excess of 0.1000*N* chromous chloride solution was added and generally the solution was allowed to stand about 1 to 2 minutes, depending on the rate of reaction, before back-titrating with standard ferric alum solutions. The titrations were performed at room temperature.

Since platinum is a catalyst for the decomposition of chromous ion by hydrogen ion, the platinum electrode was kept out of the solution until it was time to back-titrate at which time it was lowered into the solution. A similar procedure was employed with the saturated calomel electrode.

In the determination of *p*-nitrobenzeneazoresorcinol, 25 ml. of concentrated hydrochloric acid had to be used in place of the usual 10 ml., otherwise a precipitate would form.

The samples of nitroguanidine and of the monopotassium salt of acetylene dicarboxylic acid were not prepared in acid medium. The aliquots were added to enough water to give an initial volume of about 50 ml. In the presence of hydrochloric or sulfuric acid, the results were very low. In the case of nitroguanidine, the use of a citrate buffered solution did not improve the results appreciably, and in addition, the end-point response was sluggish as compared with a nonbuffered solution. In these determinations it was not necessary to allow the solution to stand for several minutes before back-titrating the excess chromous solution.

Table I. Analysis of Organic Compounds Using Chromous Chloride

	Meq. Taken	Minimum Excess, %	Meq. Found	No. of Detn.
o-Nitrobenzoic acid	0.634_5	200	0.635 ± 0.004	4
	0.903	200	0.905 ± 0.003	3
	1.269	200	1.264 ± 0.004	4
	1.806	200	1.808 ± 0.008	3
2,4,6-Trinitrobenzoic acid	0.796_5	300	0.795 ± 0.007	4
	1.593	200	1.596 ± 0.019	5
2,4,6-Trinitroresorcinol	0.511	300	0.511 ± 0.005	6
	0.635	300	0.630 ± 0.007	5
	1.022	250	1.011 ± 0.005	8
	1.270	250	1.259 ± 0.005	4
2,4-Dinitrophenyl hydrazine	0.528_5	450	0.425 ± 0.004	8
	0.857	250	0.851 ± 0.007	5
Nitroguanidine	0.452	200	0.448 ± 0.003	3
	0.501	250	0.495 ± 0.002	4
	0.904	200	0.890 ± 0.002	3
	1.002	250	0.990 ± 0.008	10
p-Nitrobenzeneazoresorcinol	0.369	250	0.371 ± 0.002	4
	0.738	250	0.731 ± 0.003	6
	0.500	250	0.493 ± 0.002	3
	1.000	250	0.995 ± 0.001	4
Nitroso R salt	0.425	300	0.423 ± 0.004	4
	0.537	300	0.536 ± 0.001	3
	0.850	300	0.850 ± 0.004	5
	1.074	250	1.074 ± 0.002	4
Monopotassium salt of acetylene dicarboxylic acid	0.517[a]	400	0.514 ± 0.004	6
	0.527_5	400	0.528 ± 0.005	5
	0.661_5	400	0.662 ± 0.002	3
	1.034[a]	250	1.025 ± 0.003	4
	1.055	250	1.055 ± 0.001	3
	1.323	250	1.325 ± 0.002	3
Anthraquinone-2,7-disodium sulfonate	1.000	...	1.006 ± 0.001	3
	1.187	...	1.194 ± 0.000	4
	2.000	...	2.015 ± 0.002	4
	2.374	...	2.396 ± 0.002	4

[a] Material synthesized according to procedure of Moureu and Bongrand.

The determination of 2,4,6-trinitrobenzoic acid had to be performed at an elevated temperature. At room temperature in either hydrochloric or sulfuric acid solution only about 70% reduction was obtained, whereas in a citrate buffered solution, the reduction was increased to about 80%. The procedure that was finally adopted involved heating a hydrochloric acid solution of the sample to 85° C. and allowing it to cool to 55° C. before back-titrating the excess chromous chloride.

A blank using the same solvent conditions as used for the samples was run on the chromous chloride for each set of reductions.

The anthraquinone 2,7-disodium sulfonate as well as an impure sample of 1-nitro anthraquinone-5-sodium sulfonate was titrated directly in a hydrochloric acid medium. The reaction at the end point was slow; therefore a 5-minute wait was allowed before making the final reading. Heating did not improve the end-point response.

RESULTS AND DISCUSSION

Table I contains the results that were obtained for the compounds investigated in this study, which indicates that satisfactory results can be obtained.

The minimum excess of chromous chloride to be added to a given sample, so as to obtain satisfactory results, varied with the nature of the compound and generally depended on its concentration in the solution. Therefore, a systematic investigation was necessary to carry out the per cent reduction for a given added excess at a particular concentration of the sample. In general, for 10 ml. of an approximately 0.1*N* solution an excess of 200 to 250% was sufficient. With smaller samples the required excess may be the same as or slightly greater than with samples of a higher concentration, or it may be considerably greater as in the case of the monopotassium salt of acetylene dicarboxylic acid or 2,4-dinitrophenylhydrazine.

The indirect determination of anthraquinone 2,7-disodium sulfonate using the general procedure invariably produced low results. This was due to the very easy oxidation of the anthrahydroquinone by ferric ion. However, the indirect determination of 1-nitroanthraquinone 5-sodium sulfonate gave the same results as the direct titration. Since the sample was impure, these data are not presented in Table I. The first appreciable voltage change (about 200 mv.) was taken to be the end point for the back-titration. After this break, there was a gradual voltage change with added ferric solution, and finally another substantial break corresponding to the oxidation of the anthrahydroquinone. This was observed qualitatively in that the reddish-brown color of the anthrahydroquinone eventually gave way to the characteristic green color of the chromic ion.

This study of the use of chromous chloride for the determination of reducible organic materials is being continued. A more thorough study of nitroso and acetylenic compounds is now in progress. An investigation will be made of the possibility of determining hydrazo compounds, diazonium salts, and certain types of carbonyl compounds, such as α-diketones, by chromous chloride reduction.

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Precise Determination of Chloride in Plasma by Differential Potentiometric Titration

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Chloride in blood plasma can be titrated directly with a differential potentiometric method. The precision of the measurement (0.1% coefficient of variation between replicates) has permitted study of small but systematic fluctuations in concentration of electrolyte. The sharp end point of the titration makes it possible to determine chloride in highly dilute solutions. The rapidity and ease of the method, once the apparatus is assembled, suggest that it might be of general value for clinical work.

DIFFERENTIAL potentiometric titration gives a more exact measure of chloride than any other method now available. MacInnes and associates (*7, 8*) reported an average difference between replicate analyses of only 0.003% on titration of large volumes of inorganic solution with two silver chloride electrodes, one of which dipped into the main body of solution and the other was enclosed in a segregated portion. A small amount of silver nitrate added to the outer solution under these conditions reduces the external concentration of chloride but leaves the segregated portion unaffected. The resultant potential between the two similar electrodes measures the rate of change of titration potential with change in concentration of chloride and has a sharp maximum at the end point. The method, although simple and rapid, has been neglected, possibly because of the special equipment required and because it was not known that it could be applied to biological mixtures without preliminary ashing.

Twelve years later Cunningham, Kirk, and Brooks (*2*) demonstrated that acidification of blood plasma suppressed the formation of silver proteinates and permitted direct potentiometric titration of chloride. Their method, however, employed bimetallic electrodes and thus registered cumulative rather than differential changes of potential on titration. Kirk (*5*) noted the possible advantages of a direct differential method for the microdetermination of halides, but left the idea for future development.

PROCEDURE

The apparatus shown in Figure 1 was designed to isolate one electrode when the glass sleeve is lowered, and to permit rapid mixing of the whole solution when the sleeve is raised. When the sleeve is lowered, the rubber ring at the foot presses onto the bottom of the beaker with the weight of the lead collar and prevents appreciable mixing of the inner and outer solutions, although the solutions remain in electrical contact through a film of electrolyte. The electrodes are made in the usual way: Silver and silver chloride are deposited electrolytically on a platinum wire sealed into the end of a glass tube (*1*). The glass shield can be used interchangeably with any electrode—an advantage over the original design of MacInnes and others. A coat of sili-

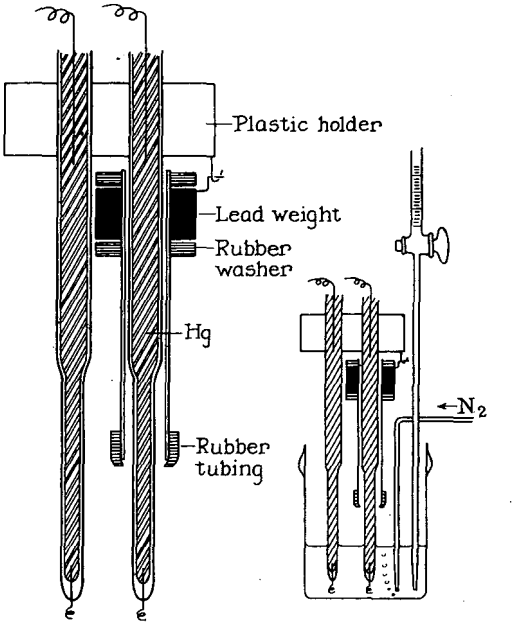

Figure 1. Titration assembly

Left. Details of electrode pair and glass sleeve
Right. Complete unit including weighing bottle, buret, and capillary for gas stirring

cone on the glass surfaces prevents creeping of solution into the space between electrode and sleeve.

The diagram at the right in Figure 1 shows the complete assembly. A buret delivers silver nitrate into the outer solution, and a second capillary carries a stream of nitrogen for stirring. (Octyl alcohol is added, if necessary, to prevent foaming.) Since the potential difference between electrodes needs to be measured only to the nearest millivolt, a student potentiometer is adequate. It is slow, however, and tedious in comparison with a vacuum tube potentiometer.

At the beginning of a titration the sleeve is raised and the zero potential checked to show any bias of the electrodes. The sleeve is lowered, trapping a small part of the solution around one electrode, and silver nitrate in suitable concentration is run into the outer solution from the buret until the potential begins to rise sharply, indicating approach of the end point. The sleeve is raised to allow mixing of the whole solution, then lowered. A small amount of silver nitrate is added; after a 30-second equilibration, the potential is read, and once again the sleeve is raised to mix the whole system. The successive values of potential rise to a maximum which can be located graphically by the intersection of lines drawn through points on either side of the end point.

When chloride is to be determined with maximum precision the samples must be measured by weight rather than by volume, since the error of pipetting a small volume is greater than that of the titration. It has proved convenient to weigh and titrate in 20-ml. flat-bottomed weighing bottles with an external ground joint for the cover (Figure 1). Three weighings are required in an analysis of serum: The titration vessel is weighed first after the addition of about 1 ml. of $1N$ sulfuric acid, again after the addition of 1 ml. of serum, and, finally, after having received 1 ml. of $0.095N$ silver nitrate (weight composition). The remaining chloride is titrated near the end point with a 1 to 5 dilution of the stock silver nitrate in increments of 0.01 ml., corresponding to about 0.2% of the total chloride present.

When speed of analysis rather than maximal precision is the objective, the sample and the silver nitrate should be measured volumetrically. The variation in delivery of plasma samples can be minimized by washing the plasma into the acid from a pipet calibrated "to contain."

THEORY

Assume that the increments in the titration are sufficiently small to give a close approximation to the true differential titration curve, that both the liquid junction potential and the activity coefficients of chloride remain constant during the titration, and that silver and chloride leave the solution in equivalent amounts—i.e., no absorption of either ion onto the precipitate. For the titration of inorganic solutions these assumptions are justified by the data of MacInnes and Dole (*7*), whose analysis of constant boiling hydrochloric acid by differential potentiometric titration gave values within 0.2% of the average value obtained gravimetrically by Foulk and Hollingsworth (*3*). With small enough increments of silver nitrate the segregation of a part of the solution causes no significant error. If the ratio of enclosed volume to free volume is 1 to 25 and if 1 ml. of serum is titrated near the end point with 0.01-ml. increments of $0.017N$ silver nitrate, the concentration of chloride in the outer solution remains within 0.005% of the value that it would have in a completely mixed system.

The potential difference between two chloride electrodes, one placed in the solution to be titrated and the second in a similar solution of arbitrary but fixed composition, is given by an expression of the form

$$E = A \log x + B \qquad (1)$$

where E is potential difference, A and B are constants, and x is the concentration of chloride ion in the variable solution, expressed in any convenient units. To simplify calculations, let x be the ratio between molar concentration of Cl^- and square root of the solubility product for silver chloride, $x = (Cl^-)/\sqrt{s}$; and let the concentration of silver ion be represented by a similar ratio, $y = (Ag^+)/\sqrt{s}$. In these units the solubility equation reduces to $xy = 1$. The total amounts of chloride and of silver in the system, expressed in the same units, will be designated by X and Y, respectively.

The curve mapped by differential titration is the derivative of Equation 1 with respect to total silver, Y:

$$E' = A\left(\frac{x'}{x}\right) \qquad (2)$$

The maximum of E', corresponding to the end point, is given by a solution of the equation

$$\frac{E''}{A} = \frac{x''}{x} - \left(\frac{x'}{x}\right)^2 = 0 \qquad (3)$$

Taking into account the solubility equation and the assumption that silver and chloride are removed from solution in equivalent amounts, then,

$$X - Y = x - y = x - \frac{1}{x} \qquad (4)$$

Differentiation of Equation 4 with respect to Y gives

$$\frac{x'}{x} = -\frac{x}{1+x^2}; \quad \frac{x''}{x} = \frac{2x^2}{(1+x^2)^3} \qquad (5)$$

Substitution of these values in Equation 3 shows the maximum of E' to be at $x = 1$, corresponding to the point of equivalence, $X = Y$.

A third differentiation of E gives a measure of the curvature at the maximum of E'. At $x = 1$, $E''' = \frac{A}{4}$ and $\frac{d^2}{d\mathrm{Ag}^2}(E') = \frac{A}{4s}$. This shows that the sharpness at the end point varies inversely with the solubility product of silver chloride. Since the addition of acid to suppress interference of protein increases this product by a factor of about 10^2 (*4*), the analysis of blood plasma cannot reach the extreme precision attainable in neutral solutions of low ionic strength (*7*). The solubility product also increases about threefold with a rise of temperature from 5° to 25° C. Lange and Schwartz (*6*) in a careful discussion of their potentiometric method recommended that titrations of chloride be carried out in an ice bath; in the present case, however, this would be of little value because the thermal effect is negligible compared with that of acidity.

RESULTS

Direct electrometric titration of chloride in acidified plasma gave the same result as the analysis of ashed samples, confirming the earlier work of Cunningham, Kirk, and Brooks (*2*). In one experiment, each of six samples from a pool of plasma was weighed into 4 ml. of $0.4N$ sulfuric acid and analyzed by direct titration. Eight other samples from the same pool were weighed into platinum crucibles, dried at 100° C. with $0.1N$ sodium bicarbonate, ashed 3 hours at 500° C., then dissolved in 4 ml. of $0.4N$ sulfuric acid, and transferred to beakers for titration. The direct analyses averaged 101.86 ± 0.098 meq. per 1000 grams, while the ashed plasmas averaged 101.82 ± 0.77 meq. per 1000 grams. Ashing merely increased the standard error, presumably because of the greater number of manipulations.

In routine use the method has given results of comparable precision. During a period of 2 months the coefficient of variation between duplicates of 50 consecutive analyses of serum was 0.16%—only slightly greater than the variation under optimal conditions.

SUMMARY

Chloride in 1 ml. of blood plasma can be determined by direct differential titration. The method gives a precise (0.1%) measure of chloride in weighed samples, more rapid analysis of volu-

metric samples, and is applicable to microanalysis of dilute solutions.

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Spectrophotometric Determination of Ruthenium

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A procedure is given for determining ruthenium by measuring the absorbance of potassium perruthenate in alkaline solution at 380 mμ. The method may be employed for amounts of ruthenium from 0.1 to 12 mg. Osmium, the only element known to interfere, may be removed by preliminary distillation.

RUTHENIUM may be determined spectrophotometrically as a thiourea complex (*1*) or as potassium ruthenate (*4*), which has an absorption maximum at 465 mμ. In the course of work on the ruthenate procedure in this laboratory, conversion to perruthenate was found to offer several advantages. Potassium perruthenate is more stable than the ruthenate and can be determined with greater sensitivity. Its absorption maximum in alkaline solution is at 380 mμ. Distillation of ruthenium (*4*) by a slightly modified method, followed by photometric determination of perruthenate, provides a reasonably rapid, sensitive, and precise procedure for the analysis of minerals and alloys containing this element.

The proposed method involves distillation of ruthenium tetroxide from a solution of perchloric acid, phosphoric acid, and sodium bismuthate containing a trace of chloride. Phosphoric acid must be present to prevent the distillation of molybdenum (*3*). The tetroxide is collected in a 2.0*M* potassium hydroxide solution. (This separates ruthenium from all interfering elements except osmium. If osmium is present in the sample it should be removed first, by distillation from a nitric acid solution.) The ruthenium distillate in alkaline solution is diluted to a known volume and its absorbance is read at 380 mμ after standing for 0.5 hour.

The mechanism of the reaction is assumed to involve oxidation of the ruthenium compound to ruthenium tetroxide by the perchlorate–bismuthate mixture, as well as the simultaneous oxidation of the chloride to free chlorine. Both the ruthenium tetroxide and the chlorine are absorbed in the potassium hydroxide solution, forming potassium ruthenate and potassium hypochlorite. The potassium ruthenate is attacked by the hypochlorite to form potassium perruthenate. The reaction is not instantaneous, however, and about 0.5 hour was found to be required for complete conversion to the perruthenate:

$$2K_2RuO_4 + Cl_2 \rightarrow 2KRuO_4 + 2KCl$$

This transformation can be detected by the color change from the orange red of the ruthenate to the greenish yellow of the perruthenate. The effect of chloride ion in the distillation is shown by comparison of curves I and II in Figure 1. Curve II presents the absorption curve of potassium perruthenate formed by the distillation of ruthenium trichloride (10 mg. of ruthenium) into alkaline solution. Curve I shows the absorption curve of potassium ruthenate formed by distilling an equivalent quantity of ruthenium sulfate without chloride but under otherwise similar conditions. A further verification of the effect of chloride ion was made by adding an excess of silver nitrate solution to ruthenium chloride prior to distillation, in order to remove all free chloride. The absorption spectrum of this distillate coincided with curve I. A small amount of chlorine was then passed through these solutions and after standing their absorption spectra were identical with curve II. The quantity of chloride ion in the sample must be carefully controlled, since hypochlorite absorbs strongly at 296 mμ and large amounts of hypochlorite will interfere with the 380-mμ perruthenate peak. Figure 1 indicates that the perruthenate peak is much more sensitive

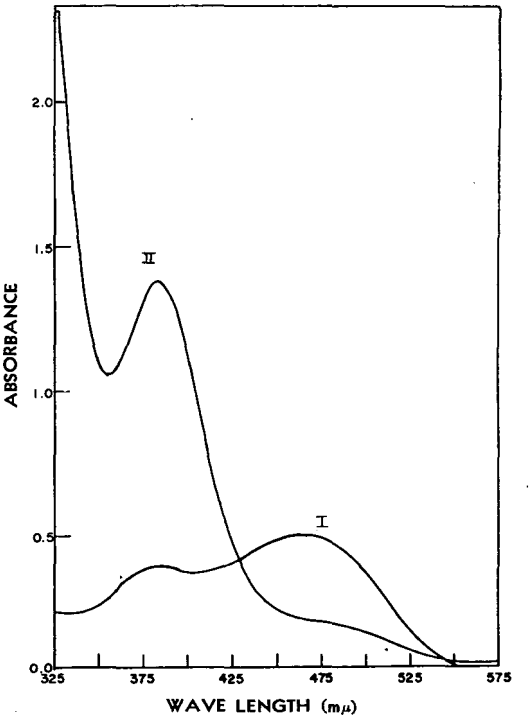

Figure 1. Spectrophotometric curves

I. Potassium ruthenate, 10 mg. of ruthenium per 100 ml.

II. Potassium perruthenate, 10 mg. of ruthenium per 100 ml.

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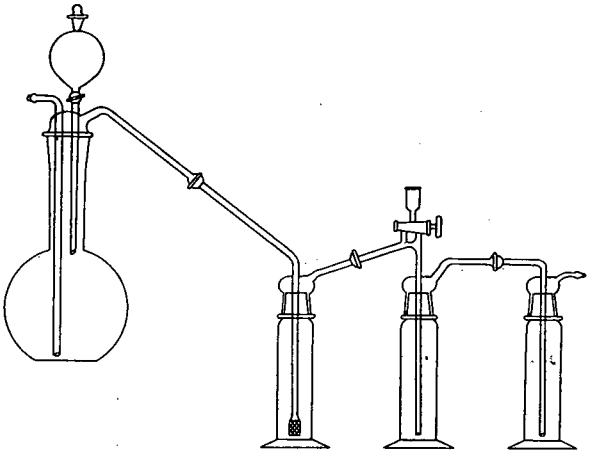

Figure 2. All-glass distilling apparatus for ruthenium tetroxide

than the 465-mμ ruthenate peak. The perruthenate solutions were found to be stable even after overnight standing or boiling, whereas potassium ruthenate solutions are not stable over long periods.

EXPERIMENTAL

Reagents. Ruthenium trichloride from the American Platinum Works was dissolved and standardized.

Osmium tetroxide was obtained from the Fisher Scientific Co.

Sodium bismuthate, ACS reagent.

Potassium hydroxide solution, 2*M*. Dissolve 112 grams of reagent grade potassium hydroxide in 1 liter of water.

Hydrochloric acid, sulfuric acid, nitric acid, sodium carbonate, and sodium bisulfate were ACS reagent.

Perchloric acid, 70%, reagent grade.

Phosphoric acid, 85%, reagent grade.

Apparatus. During the development of this method all spectral curves were recorded on a Cary recording spectrophotometer.

A standard calibration curve was prepared from absorbance measurements made on a Beckman Model DU spectrophotometer using 1.001-cm. matched Corex cells and with water as the reference solution. Its linearity indicated excellent agreement with Beer's law. All distillations were carried out in the special all-glass distillation apparatus illustrated in Figure 2. The distillation flask has a capacity of 500 ml. and the receivers of 125 ml. each.

Preparation and Standardization of Ruthenium Solutions. Ruthenium trichloride was dissolved in water which contained 1% (by volume) of hydrochloric acid. The solution was then diluted to contain approximately 2 mg. of ruthenium per ml.

This solution was standardized by pipetting a known amount of solution into a tared porcelain boat and evaporating slowly to dryness on a hot plate. The boat was allowed to cool and then placed in a Vycor combustion tube. Hydrogen gas was passed through the tube for 15 minutes to remove all the air from the tube and the boat was ignited in the hydrogen atmosphere at the full heat of a Meker burner to constant weight. Duplicate samples were analyzed and found to agree very well. The solution which was used for the preparation of the standard curve had a concentration of exactly 2.00 mg. of ruthenium per ml.

Preparation of Calibration Curve. Suitable aliquots of the standard ruthenium solution were pipetted into 500-ml. distilling flasks and diluted with 10 ml. of water. The distilling train was assembled with each of the absorption bulbs containing 50.0 ml. of 2.0*M* potassium hydroxide. The second absorber is used only as a precaution. If the solution in it turns yellow during distillation, some ruthenium tetroxide is escaping the first bulb. This has never been observed in the tests conducted so far.

One gram of sodium bismuthate, 1 ml. of phosphoric acid, and 10 ml. of perchloric acid were added as rapidly as possible; the distilling head was inserted immediately, and air was bubbled through the train at a rate of about 2 bubbles per second. The flask was then heated and the distillation mixture allowed to boil gently for 0.5 hour or until distillation was complete. Then the first absorber was removed, its contents were transferred to a 100-ml. volumetric flask, and the solution was brought to volume with water. After a thorough mixing the solution was allowed to stand at least 0.5 hour and its absorbance was read at 380 mμ. The data obtained from the series of standards yielded a straight line when plotted.

Table I. Determinations of Ruthenium Added to 2.0 Grams of Calcareous Shale

(Fused with 7.0 grams of sodium carbonate)

Sample	Ru Added, Mg.	Absorbance	Ru Recovered, Mg.	Error, Mg.
1	0.00	0.006	0.01	....
2	2.00	0.273	2.00	−0.01
3	3.00	0.400	2.95	−0.06
4	4.00	0.543	4.03	+0.02
5	6.00	0.800	6.00	−0.01
6	8.00	1.08	8.07	+0.06
7	10.00	1.35	10.08	+0.07

PROCEDURE

Preparation of Sample, Osmium Absent. If the sample is an alloy, dissolution in hydrochloric or nitric acid is usually necessary. Dissolve a sample of suitable size in a minimum of nitric or hydrochloric acid and filter off any insoluble material using a close textured paper (Whatman No. 42). Transfer the insoluble material and paper to a clean platinum crucible. Dry, burn off the paper, and ignite at 950° C. for 0.5 hour. Cool the crucible, and fuse the residue with sodium bisulfate at 400° C. Dissolve the fusion melt in the original filtrate with sulfuric acid or until the final solution is strongly acid, and evaporate to fumes of sulfur trioxide. This step is necessary to remove all hydrochloric and nitric acid.

If the sample is a silicate rock or mineral in which a basic fusion is necessary to effect solution, fuse the sample with sodium carbonate at 1000° C. for 1 hour in a platinum crucible. Then dissolve the melt in water and sulfuric acid (1 ml. of acid for each gram of sodium carbonate in the fusion melt).

Distillation. Transfer the sample as prepared to the distilling flask (Figure 2). Add 5 drops of concentrated hydrochloric acid, 1 ml. of phosphoric acid, 1 gram of sodium bismuthate, and 10 ml. of 70% perchloric acid. Rapidly attach the flask to the absorption train. Pass air slowly through the train, heat the solution to a gentle boil, and continue the distillation for at least 0.5 hour. When distillation is complete detach the absorption bulb, and transfer its contents to a 100-ml. volumetric flask diluting to volume with water. Allow the solution to stand 0.5 hour or longer and read its absorbance at 380 mμ. Compare the reading with the standard curve and calculate the per cent of ruthenium.

Alternative Procedure if Osmium Is Present. In the presence of osmium the solution step is modified to remove the osmium prior to distillation of ruthenium tetroxide. After solution of the sample is complete, add 25 ml. of concentrated nitric acid and boil gently while bubbling a slow stream of air through the solution. One hour is usually sufficient to remove osmium when it is present as the osmate or bromoosmate. However, when it is present as the chloroosmate, 7 or 8 hours are said to be required (*3*). After removal of the osmium as the tetroxide, add sulfuric acid, evaporate to fumes of sulfur trioxide, and proceed with the bismuthate distillation as already described.

APPLICATIONS

The method was applied to the determination of approximately 0.1% ruthenium in a uranium alloy. The alloy also contained small amounts of molybdenum, neodymium, and zirconium. Duplicate results agreed within 0.002%. Several sedimentary rock samples were also analyzed. Their ruthenium contents were found to be negligibly small, but known additions of ruthenium could be recovered almost quantitatively, as indicated in Table I. The results of such work indicate that this method should be adaptable to both mineral and alloy analysis.

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Application of Volhard Titration to 2-Ethyl-1-Hexanol Separation Method for Determination of Lithium

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The Volhard titration method for the determination of chloride has been applied successfully to the determination of lithium as lithium chloride following its separation from sodium and potassium chlorides by extraction with 2-ethyl-1-hexanol. The titration of chloride is conducted directly in the alcoholic phase after a single extraction. The method is applicable in the range of 1 to 50 mg. of lithium; the total quantity of chloride taken should not exceed 500 mg. The method also provides a substantial savings in time of analysis at no sacrifice in precision, which is within 0.5%.

THIS investigation was undertaken to develop a rapid yet precise method for the determination of lithium in the presence of sodium and potassium. The most widely used method is based on the solubility of lithium chloride, in contast to the slight solubility of sodium and potassium chlorides, in higher aliphatic alcohols (*4*), in particular 2-ethyl-1-hexanol (*2*), thereby precipitating sodium and potassium as the chlorides. The precipitate is separated by filtration through a sintered-glass crucible and the lithium chloride determined either by washing the residue with alcohol, drying, and then weighing the combined sodium and potassium chlorides; or by converting the alcoholic solution of lithium chloride to lithium sulfate and weighing it as the sulfate. This method provides an excellent means of separating lithium from the other alkali metals. The determination of lithium in the alcoholic phase is, however, at best, tedious and time-consuming because of the care required in evaporating the high-boiling 2-ethyl-1-hexanol and converting the chloride to the sulfate salt.

Table I. Effect of Extraction Time on Determination of Lithium

(By extraction of lithium chloride with 2-ethyl-1-hexanol sample composition; 4.96 mg. of Li, 10 mg. of Na, and 150 mg. of K)

Extraction Time, Min.	Lithium, Mg. Av.	Lithium, Mg. Std. dev.	Coeff. of Variation, %
0	4.97	0.03	0.7
1	4.94	0.03	0.7
5	4.94	0.03	0.7
10	4.93	0.03	0.7
15	4.94	0.03	0.7

The successful application of the Volhard (*5*) method is described for the titration of chloride to the determination of lithium chloride in the alcoholic solution which is obtained following extraction, thereby materially decreasing the over-all time and cost of the determination.

REAGENTS

Standard Solution of Lithium Chloride, 5 mg. of lithium per ml. Prepare pure lithium carbonate by the method of Caley and Elving (*3*). Dissolve carefully 26.29 grams of the purified salt in 1 to 5 hydrochloric acid, boil to remove carbon dioxide, and dilute to 1 liter. Calculate the exact concentration of lithium in solution.

Sodium Chloride Solution, approximately 10 mg. of sodium per ml. Dissolve 25 grams of reagent grade sodium chloride in water and dilute to 1 liter.

Potassium Chloride Solution, approximately 100 mg. of potassium per ml. Dissolve 165 grams of reagent grade potassium chloride in water and dilute to 1 liter.

2-Ethyl-1-hexanol. Distill and retain the fraction boiling between 179° and 182° C.

Ethyl alcohol, reagent grade, 95%.

Nitric Acid, concentrated.

Standard Silver Nitrate, 0.1*N*. Dissolve 17 grams of silver nitrate, dried at 120° C., in water and dilute to 1 liter. Standardize against pure sodium chloride.

Standard Potassium Thiocyanate, 0.1*N*. Dissolve 10 grams of potassium thiocyanate in water and dilute to 1 liter. Standardize against the solution of standard silver nitrate.

Ferric Indicator. Dissolve 20 grams of ferric ammonium sulfate in 100 ml. of water.

PROCEDURE

Transfer an aliquot, not larger than 20 ml., containing 5 to 25 mg. of lithium in the form of the chloride, and not more than 500 mg. of total alkali metal chlorides, free from other cations, to a 50-ml. Erlenmeyer flask and gently take to dryness. Bake to ensure removal of excess hydrochloric acid. Dissolve the salts in 3 to 5 ml. of water. Add 10 to 15 ml. of 2-ethyl-1-hexanol and one or two glass beads. Heat slowly on an electrically controlled heater until the temperature of the solution is about 135° C., and the aqueous phase is completely volatilized. Continue heating at 135° C., until the bulk of the salts that crystallize out of solution becomes free-flowing and no longer clings to the walls of the vessel, then for 3 additional minutes. Cool, then filter the solution through a sintered-glass crucible of medium porosity. Wash with 1- to 2-ml. volumes of cold 2-ethyl-1-hexanol and catch the filtrate in a titration flask of 250-ml. capacity. Add 50 ml. of ethyl alcohol to the filtrate, then cautiously add 2 ml. of concentrated nitric acid and 2 ml. of ferric indicator. To the flask, add 1 ml. of potassium thiocyanate solution and titrate, with continuous swirling, until about 0.5 ml. of silver nitrate in excess has been added. The excess is noted by fading of the iron-thiocyanate complex. Stopper the flask, shake vigorously, then allow the precipitate to settle. Swirl gently, then titrate with thiocyanate, dropwise, until a faint, permanent pink color develops. If phases separate, add additional alcohol. The silver nitrate consumed by the chloride is derived by subtracting the volume of the thiocyanate solution multiplied by the ratio of silver nitrate to thiocyanate from the volume of silver nitrate used. A volume of 1 ml. of 0.1*N* silver nitrate is equivalent to 0.694 mg. of lithium.

RESULTS

Extraction Time. Experiments were confined to a single extraction of lithium chloride with 2-ethyl-1-hexanol. Caley (*2*) reported poor results when an attempt was made to separate 100 mg. of lithium chloride by a single extraction. He also showed that dehydration of the solution at too high a temperature, or prolonged boiling of the 2-ethyl-1-hexanol phase, produced hydrolysis of the lithium chloride and subsequent low recoveries of lithium through loss as slightly soluble lithium hydroxide. In order to avoid double extractions, the amount of lithium chloride involved was maintained at a level below 100 mg. The concentration of sodium and potassium chlorides was set at approximately 35 times the weight of lithium taken and a maximum of approximately 400 to 500 mg. of chloride salts was subjected to extraction. The extraction time was measured from the

point at which the bulk of the insoluble chlorides became free-flowing and no longer clung to the walls of the flask.

A chloride solution containing 4.96 mg. of lithium, 10 mg. of sodium, and 150 mg. of potassium was taken as the standard solution. This described procedure was followed but the extraction time was varied from 0 to 15 minutes. The temperature of the extraction medium was controlled at 135° ± 5° C. by an electric heater. Four determinations were made at each extraction time. The results are shown in Table I.

These test results clearly show that the extraction of lithium chloride with 2-ethyl-1-hexanol is essentially complete as a result of contact during the dehydration period and that further contact is no longer necessary once the insoluble salts become free-flowing. Continued contact after this state is not detrimental, at least for periods of time up to 15 minutes, at temperatures below 140° C., but is of course completely unnecessary. Since the point at which the bulk of the insoluble salts become free-flowing is subject to interpretation by the observer, extraction should be continued for approximately 3 minutes beyond this stage to ensure complete extraction, and more important, to ensure complete insolubility of the sodium and potassium salts.

The optimum conditions for extraction were also investigated. In the first trials the chloride salts were taken to dryness and the extraction carried out on the dried salts. It was necessary to break up the crystals so that adequate contact was attained during extraction. Results which were obtained by this technique were low and erratic (coefficient of variation, 2% for 5 mg. of lithium). To avoid the formation of large crystals, the dried chlorides were dissolved in a minimum of water, ethyl alcohol was added to the point of saturation, and finally the volume of 2-ethyl-1-hexanol was added. The salts which are insoluble in this solution were finely divided and extraction proceeded satisfactorily. Excellent results (coefficient of variation, 0.7% for 5 mg. of lithium) were obtained. Subsequent tests proved that the addition of ethyl alcohol was superfluous. The final procedure, is based on these observations.

Table II. Comparative Results of Modified Method

(Applied in presence of aqueous organic solutions of lithium chloride)

	Lithium, Mg.		Coefficient of Variation, %
	Taken	Found[a]	
Water leach	4.96	4.94	0.03
Organic phase	4.96	4.94	0.03

[a] Average of four determinations.

Application of Volhard Titration of Chloride. The greatest difficulty with the determination of lithium arises from the conversion of the alcoholic solution of chlorides to sulfate. To circumvent this step, the possibility of titrating the chloride ion of the extracted lithium chloride was investigated. The Volhard (*5*) method appeared to be the most promising of the available titration methods. Initial tests consisted of washing the organic extract with water to which a few drops of nitric acid had been added to prevent emulsification. Subsequent tests consisted of conducting the titration directly in the organic phase. Ethyl alcohol was used to dilute the solution. The addition of nitrobenzene, as an agglomerating agent (*1*) to prevent dissolution of silver chloride, was eliminated because of the solubility of nitrobenzene in ethyl alcohol. Possible error as a result of this modification was reduced since silver chloride is less soluble in alcohol than in water (*6*). A comparison of the results obtained by water leaching of the 2-ethyl-1-hexanol extract and by direct titration in the organic phase is shown in Table II.

The method was also tested on a group of samples which contained known amounts of lithium chloride and varying concentrations of potassium and sodium chlorides. In all cases the total amount of chloride salts was less than 500 mg., thus making it possible to utilize only a single extraction. The results are shown in Table III.

Table III. Determination of Lithium in Presence of Sodium and Potassium Chlorides

(Modified 2-ethyl-1-hexanol extraction method)

Taken, Mg.			Av. Found, Mg.		Coefficient of Variation, %
Sodium	Potassium	Lithium (A)	Lithium (B)	Difference B − A	
1	15	0.496	0.585	−0.089	100
2	30	0.992	0.984	−0.008	0.35
150	10	4.96	4.93	−0.03	0.30
20	130	9.92	9.86	−0.06	0.45
20	60	24.8	24.6	−0.2	0.35
5	25	49.6	49.3	−0.3	0.20

The data indicate an excellent order of precision but also show a slight negative bias of 0.6%. The cause of this bias is not the result of incomplete extraction of lithium chloride because the presence of lithium in the residue was not detected by the flame photometer. More probably it is the result of an error in standardization of the lithium salt. Such a bias can easily be corrected by following the process of standardization of the standard solutions of silver nitrate against standard solutions of lithium chloride.

The applicable range of lithium in this determination is from 1 to 50 mg. When lower amounts are taken the results are poor, as shown in Table III, for 0.5-mg. quantities. In the experiment with 0.5 mg. of lithium, extraction of lithium chloride from the sodium and potassium salts was quantitative because the analysis of the filtrate by flame photometer revealed that neither cation was present. Titration of the chloride is not satisfactory at this low concentration because the silver chloride formed by addition of silver nitrate exists as a suspension and detection of the end point is difficult. When the total quantity of chlorides exceeded 500 mg., a single extraction with 2-ethyl-1-hexanol was not adequate. For example, when 10 mg. of lithium was determined in the presence of 20 mg. of sodium and 300 mg. of potassium (total weight of salts, approximately 750 mg.) the recovery of lithium was only 90% complete and was not reproducible. Prolonged extraction in excess of 15 minutes resulted in hydrolysis of lithium chloride to insoluble lithium hydroxide.

The order of precision attainable by this method is superior to those obtained by conversion to sulfate or water leaching. The best precision for these methods was in excess of 1% under optimum conditions.

The method also fulfills the requisite of speed. For an 8-sample block, starting with the chloride salts, approximately 1 hour is required for analysis.

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Location of Symmetric Peaks by a Simple Least Squares Method

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A simple expression is developed for computing the location of a symmetric maximum or minimum. The expression, based on the orthogonal polynomial method of least squares, eliminates most of the intermediate work and markedly shortens the time for computation. The formula is derived for seven equally spaced intervals of the independent variable.

IN MANY instances of spectrometric work the location of the point of maximum emission or absorption in terms of wave length is desired. For example, x-ray spectrometry (fluorescence analysis) requires determining the peak position of the x-ray lines being measured. While the 2θ-Bragg angle may be calculated from the known wave length of the line and the "$2d$ spacing" of the analyzing crystal, determination of the exact peak position empirically is advisable. First, the initial alignment of the goniometer requires doing this for one element, generally copper. Second, slight lack of parallelism between the physical surface and the corresponding crystal plane will cause other peaks to be somewhat displaced even if the aligned peak is perfect. There are other reasons for the desirability of precise peak location in other types of work. The following method was developed specifically for x-ray spectrometry, but can be applied to any case where the peak is fairly symmetric, and where the independent variable may be chosen at equally spaced intervals.

PRINCIPLE

For a symmetric peak the measurement (intensity, absorbance, etc.) may be fitted to an empirical equation

$$y = a + bx + cx^2 \tag{1}$$

where x is the independent variable in arbitrary units. Because of the method to be applied, it is desirable to have the variable, x, in arbitrary units which designate the lowest value as zero, the next one, the next two, and so on. The relationship between the arbitrary units, x, and the usual units—i.e., wave length—can be expressed by $P = P_o + x\,\Delta P$. Here P is the usual unit, P_o is the smallest value of P used, and ΔP is the size of the equal interval. The peak position, either a maximum or a minimum, may be determined if the constants of Equation 1 are known by setting the first derivative of y equal to zero. For precise determination of the constants, a least squares solution is desirable and can be effected without great labor. For frequent determination of peaks, the computational labor can be greatly reduced by prior algebraic solution of the data by the least squares orthogonal polynomial method (*1*). This method has been applied to Equation 1 in order to obtain a simple analytical expression for the peak position. A brief outline of the derivation is given here together with the analytical expression so derived. If the experimenter can choose his conditions to fit those required here, and this is often possible, the procedure outlined will give a good peak location with a minimum of computational labor.

The method of orthogonal polynomials replaces Equation 1 with

$$y = A + BX_1 + CX_2 \tag{2}$$

where A, B, and C are new constants and are obtainable by the method of orthogonal polynomials applied to the observed values. [The reader interested in the orthogonal polynomial method per se should read Snedecor (*1*).] In Equation 2

$$\left.\begin{aligned} X_1 &= x - \bar{x} \\ X_2 &= X_1^2 - \frac{n^2 - 1}{12} \end{aligned}\right\} \tag{3}$$

In Equation 3, n is the number of observations. These observations must be equally spaced in order to apply the method. For example, readings may be taken at wave lengths of 460, 470, 480, 490, 500, 510, and 520 mμ. Furthermore, x is now expressed as the number of equally spaced units beyond the first. For the wave lengths just quoted as an example, x would be 0, 1, 2, 3, 4, 5, and 6, respectively. Note that $\bar{x}$ is the average. Equating to zero the first derivative of y with respect to x yields

$$\frac{dy}{dx} = B\frac{dX_1}{dx} + \frac{dX_2}{dx} = 0$$

Therefore

$$B + 2C(X_1)_m = 0$$

and

$$x_m = \bar{x} - \frac{B}{2C} \tag{4}$$

where the subscript m indicates the value of x or X_1 for which y is a maximum or minimum. Since now B and C are simple additive functions of the observed y's, Equation 4 leads to particularly simple expressions for the peak position. If B and C are replaced by their equivalents in terms of the sums of the orthogonal polynominal method, the desired expression may be obtained. The result for seven observations is noted in step 7 of the procedure.

Table I. Calculation of Experimental Cobalt Kα Peak

Step 1 (Data)		Steps 2 and 3			
2θ, °	t, sec.	x	$10(t - 36.2)$ I	4 and 5 II	6 III
53.43	38.6	0	24	24	24
53.44	37.7	1	15	39	63
53.45	36.5	2	3	42	105
53.46	36.2	3	0	42	147
53.47	36.7	4	5	47	194
53.48	36.8	5	6	53	247
53.49	37.1	6	9	62	309
			$S_1 = 62$	$S_2 = 309$	$S_3 = 1089$

Step 7. $$x_m = \frac{60(62) - 51(309) + 12(1089)}{24(62) - 18(309) + 4(1089)}$$

$$x_m = 3.649$$

Step 8. $(2\theta)_m{}^a = 53.43 + 3.649(0.01)$

$= 53.466°$

[a] The $(2\theta)_m$ corresponds to P_m of Equation 6 and refers to the value of 2θ for which the time to accumulate 25,600 counts is a minimum and for which, therefore, the intensity is a maximum.

PROCEDURE

1. Take 7 equally spaced observations of y near the peak for which the position is to be determined.
2. Set the y's in an ordered column with that corresponding to the lowest x first. A constant may be subtracted from all readings and a constant multiplier used to give a new set of numbers of lower magnitude without decimal, if so desired. This is called "coding."
3. Sum the (coded) y's. This is S_1.
4. Write another column with the first member the same as that in column I. The second member of the new column is the sum of the first member of the new column and the second mem-

ber of the first column. The third member of the new column is the sum of the second member of the new column and the third member of the first column. Thus the mth member of the new column is the sum of the preceding member of the new column and the mth nember of column I.

5. Summing column II gives S_2.
6. A third column is obtained from column II just as column II was obtained from I. The sum of column III is S_3.

7. Calculate $x_m = \frac{60S_1 - 51S_2 + 12S_3}{24S_1 - 18S_2 + 4S_3}$. (5)

8. The peak position is given now by

$$P_m = P_o + (x_m)(\Delta P) \quad (6)$$

where P_o is the position of the first reading, x_m is calculated according to step 7, and ΔP is the size of the equally spaced interval in the usual units.

EXAMPLE

The intensity of x-ray spectrometer lines is measured precisely by measuring automatically the time required to accumulate a fixed number of counts. The number of counts per unit of time is then the measure of intensity. Determining a peak position may be done by taking measurements at equal intervals of 2θ about the expected point. (For this type of measurement, P is 2θ.) If the intensity is a maximum, the time is a minimum. Such times were determined for the cobalt Kα line by the following data taken with a lithium fluoride analyzing crystal. The various steps are indicated.

Observations other than seven are permissible but the expression for x_m is not as simple as that shown in step 7. The corresponding expressions may be derived by carrying out the standard orthogonal polynomial method. The expressions for x_m when n = 5, 6, 7, 8, or 9 were all computed and that for seven points was the simplest. It must be emphasized that the peak must be symmetric in the form used. By decreasing ΔP and limiting the measurements to a small region around the peak, departure from symmetry may sometimes be reduced to a negligible magnitude. For the illustrative data (Table I), the orthogonal polynomial method was used to determine that the cubic and further terms beyond x^2 were not statistically significant.

The usefulness of the method consists in its simplicity which results in a great saving of time. For example, these data were computed in about 4 minutes. Using the same data but the more usual method involving squaring, cubing, etc., took 20 minutes. If uneven intervals had been used, the long method would probably have taken from 30 to 60 minutes.

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Routine Exchange Capacity Determinations of Ion Exchange Resins

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Simple, rapid methods for the determination of both anion and cation exchange capacities of ion exchange polymers have been devised using equipment generally available in all laboratories. Routine differentiations between acidic or basic groups of varying degrees of dissociation have been extended to include the expanded spectrum of functional groups currently available. The effect of the initial ionic form of the resin on the measured capacity has also been determined.

THE extensive use of ion exchange resins in chemistry has increased the necessity for methods by which their chemical properties can be accurately and rapidly measured. Although a number of such methods, designed for a particular resin or exchange system, have been suggested, little information has been published concerning the applicability of these methods to the variety of ion exchange resins now commercially available.

In routine work, the most easily measured chemical property of an ion exchange polymer is the number of groups capable of entering into ion exchange reactions. This measurement, expressed as milliequivalents of exchangeable ion per dry gram of polymer, is commonly referred to as the cation or anion exchange capacity of cation and anion exchanging polymers, respectively. In addition, these groups can be characterized as either weakly or highly dissociated acids or bases.

Although the determination of the total exchange capacity may appear to be simple, many factors complicate the determination, such as low rates of exchange and diffusion, unfavorable exchange equilibria between certain pairs of ions, inaccessibility of certain exchange sites, and instability of certain ion exchange polymers. In studying the chemical properties of ionic polymers, only the equilibrium pH titration curve (*3*) gives information which can be used to determine the effect of polymer structure on the chemical behavior of the polymer. This method requires the setting up of a series of samples in contact with varying amounts of the ion to be exchanged and frequently takes from 1 week to 6 months for the establishment of equilibrium. In certain cases similar information may be obtained by the direct titration of the polymer in either the hydrogen or the hydroxide form in the presence of a neutral salt (*1*, *2*). This titration is not successful, however, unless the resin functional groups are highly ionized as in the case of sulfonic or quaternary ammonium groups. The polymer sometimes must be ground to obtain a satisfactory rate of exchange in such direct titrations, thereby introducing additional uncertainty of changing the chemical as well as the physical properties of the polymer.

To overcome the difficulties inherent in either titration method, a series of techniques has been developed involving the exchange of one ion, present in large excess in solution, for another ion on the resin sites. The latter ion is chosen so that the resin can be easily and completely converted to that form, exchanged for the ion present in excess in solution, and determined readily by titration after the exchange reaction is complete. Methods of this type were first presented by Kunin and Myers (*3*). Since the initial work, however, the development of new resin types has necessitated the modification of many of these methods so that they may be applied more widely. These modifications and the more pertinent data leading to their development are outlined here.

APPARATUS

The simple apparatus shown in Figure 1, consisting of a short-stemmed 60° funnel supported in a 1-liter volumetric flask, is the

most practical for routine capacity determinations. A filter paper of medium porosity is used.

CATION EXCHANGE CAPACITY

The number of groups capable of exchanging cations is conveniently determined by converting the resin groups to the hydrogen form with an excess of acid, rinsing to remove this excess acid, and equilibrating the resin with a known excess of standard sodium hydroxide.

Procedure. Place approximately 5 grams of resin in the funnel of the apparatus (Figure 1). Convert this sample to the hydrogen form with 1 liter of 1*M* nitric acid. Rinse the resin free of excess acid and drain.

Weigh an approximately 1.0-gram (nearest mg.) sample of the resin prepared above into a dry 250-ml. Erlenmeyer flask. Use the remaining sample to determine the solids content, drying at 110° C. overnight. To the sample in the Erlenmeyer flask add exactly 200 ml. of standardized 0.1*N* sodium hydroxide solution that has been prepared in 5% sodium chloride. Allow the stoppered sample to stand overnight. Back-titrate 50-ml. aliquots of the supernatant liquid to the phenolphthalein end point with standard 0.1*N* acid. The cation exchange capacity is calculated as follows:

$$\frac{(200 \times N_{NaOH}) - 4(\text{ml.}_{acid} \times N_{acid})}{\text{sample wt.} \times \frac{\%\ \text{solids}}{100}} = \frac{\text{meq. cation exchange capacity}}{\text{gram of dry H-form resin}}$$

Nitric acid is recommended for the conversion of the resin to the hydrogen form as it more conveniently regenerates certain heavy metal forms of cation exchangers that precipitate with chloride or sulfate ions. The resin is converted to the acid form before weighing to eliminate errors arising from differences in the equivalent weights of different ionic forms. The use of deionized or distilled water in the rinse step is important because many resins are capable of exchanging hydrogen ions for the cations in tap water, thereby giving rise to long rinses and low capacities. Sodium chloride is added to the standard sodium hydroxide to drive the exchange equilibrium for weakly acidic resins to completion. Table I illustrates the effect of sodium chloride concentration on the measured capacities of resins of varying functionality. The 5% concentration of sodium chloride was chosen for a routine procedure not only because the values obtained with it agreed most closely with the theoretical values obtained from polymer composition but also to conserve reagents where large numbers of samples are to be analyzed. Results, for a given polymer, are equally reproducible at the 5 or 10% salt concentration and in certain research situations the use of 10% or more sodium chloride may be indicated. The method is capable of reproducibility of at least 1 part per 100 in routine application.

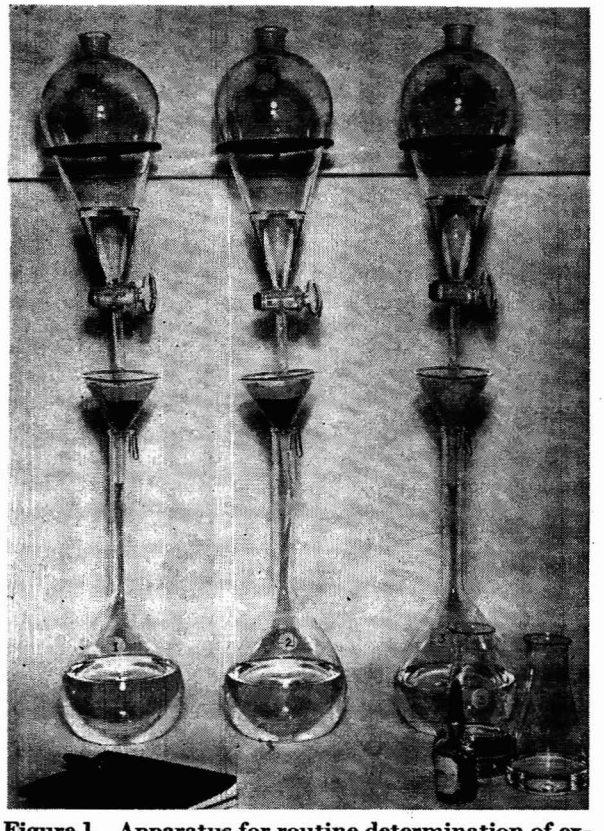

Figure 1. Apparatus for routine determination of exchange capacities of ion exchange polymers

Table I. Variation of Cation Exchange Capacity with Concentration of Sodium Chloride in Sodium Hydroxide

Resin Type	Capacity, Meq. per G.				
		Determined, % NaCl			
	Theoretical	0	1	5	10
Carboxylic I	10.6	10.12 10.12	10.24 10.24	10.49 10.38	11.18 11.03
Carboxylic II	12.2	11.50 11.58	11.80 11.76	12.11 12.06	12.63 12.40
Carboxylic Sulfonic		5.12 5.08	5.23 5.19	5.57 5.52	5.69 5.72
Phosphonic	8.7[a]	8.73 8.80	8.91 8.91	8.86 8.72	8.79 8.68

[a] From titration curve.

Table II. Effect of Resin Form and Regenerant Acid on Salt-Splitting Cation Capacity

Resin Form	Salt-Splitting Cation Capacity, Meq. per G.	
	1*M* HCl regeneration	1*M* HNO_3 regeneration
Fe^{+++}	4.23	4.49
Hg^{++}	4.39	4.49
Ba^{++}	2.43	4.04[a]
Pb^{++}	2.21	4.49
Ag^{+}	3.08	4.49
Na^{+}	4.49	4.49

[a] 4.42 meq./g. with 2 liters of 1*M* HNO_3 as regenerant.

HIGHLY DISSOCIATED CATIONIC GROUPS

In many instances wherein the characterization of ion exchange polymers is attempted, a differentiation between cation exchanging groups whose hydrogen forms are ionic and those whose hydrogen forms are largely undissociated is desired. The outlined capacity determination measures the sum of both weakly and strongly dissociated groups. A clear-cut differentiation between the two types of groups can only be obtained when they vary greatly in their degree of ionization, but an estimation of the number of highly dissociated groups may be obtained by taking advantage of the equilibrium reaction

$$\text{Resin H} + \text{Na}^+ \leftrightharpoons \text{Resin Na} + \text{H}^+$$

This reaction may be driven toward completion to the right by a large excess of sodium ions if Resin H is highly ionized. Under the same conditions the extent of reaction of weakly acidic groups, such as a carboxyl group, is 0 to 10% depending on their acid strength.

Procedure. In determining the concentration of highly dissociated cationic groups, the resin is first converted to the hydrogen form and rinsed as for the determination of cation exchange capacity. A 5.0-gram (to nearest 5 mg.) sample is weighed into a funnel of the apparatus shown in Figure 1. At the same time a sample is weighed for a solids determination. The capacity sample is leached with exactly 1 liter of 4% neutral sodium sulfate. Aliquots of 100 ml. are taken for titration with standard 0.1*N* sodium hydroxide using phenolphthalein as the indicator. Results are calculated as milliequivalents per gram dry resin as follows:

$$\frac{\text{Ml.}_{NaOH} \times N_{NaOH} \times 10}{\text{sample wt.} \times \frac{\%\text{ solids}}{100}} = \frac{\text{meq. of strong acid capacity}}{\text{gram of dry H-form resin}}$$

This method has a reproducibility of 5 parts per 1000 by a single analyst with agreement of 1 part per 100 between analysts. Typical results, illustrating the need for nitric acid as a regenerant, are given in Table II. In these experiments, 5.0-gram samples from a large batch of sodium-form sulfonic resin (Amberlite IR-120) were weighed out, converted to the indicated forms with an excess of the respective nitrate solution, and then regenerated using hydrochloric and nitric acids. Separate samples were used for the two regenerations. Where a resin is known or suspected to be exhausted with heavy metal ions, care must be exercised in the regeneration step or low capacity results in both determinations will result. A sulfonic resin was chosen for this study because it is known to be more difficult to reconvert to the hydrogen form than weakly acidic polymers.

ANION EXCHANGE CAPACITY

The anion exchange capacity can be determined by converting the resin to the hydroxide form, rinsing free of excess hydroxide, and subsequently equilibrating the resin with an excess of standard acid in a manner analogous to that used in the determination of cation exchange capacity. However, inaccuracies arise from the drying of the hydroxide form of some anion exchange resin in the solids determination and from the introduction of carbonate in the preparation and rinsing of the resin. With quaternary resins, the conversion of the material to the hydroxide form is not easily accomplished when the resin to be analyzed is in a variety of ionic forms. The data accumulated in Table III were obtained by taking 10-gram portions of the chloride form of a given batch of Amberlite IRA-400, converting them to the indicated forms with an excess of the sodium or potassium salt of the desired anion, regenerating each portion with 1 liter each of 1*M* sodium hydroxide, rinsing with deionized water, and finally determining the hydroxide content of the resin phase by elution with neutral salt by the method of Kunin and Myers (*3*). All results are based on the dry weight of the original chloride form resin. The complete data obtained form a replacement series for these ions and hydroxide.

The difficulty with which many ionic forms were converted back to the hydroxide form led to an investigation of other means of determining the capacity of anion exchange polymers. An anion exchange method, wherein the resin had been converted to the chloride form, rinsed and subsequently eluted with sodium sulfate, had also been proposed (*3*). This method had been modified to provide an estimation of both weakly and strongly basic groups as well as the total anion capacity of the polymer. Weakly basic materials are easily regenerated by dilute ammonia solutions while more strongly basic groups are regenerated only partially or not at all.

Procedure. Transfer approximately 10 grams of the material to be analyzed to the funnel of the apparatus in Figure 1. Convert the resin to the chloride form by passing 1 liter of 1*M* hydrochloric acid through each sample. Rinse with alcohol until the effluent is neutral to methyl orange. Weigh 5.0 grams (to nearest 5 mg.) of the prepared chloride-form resin into a fresh funnel. Use the remaining portion for a solids determination. Leach the capacity sample with exactly 1 liter of 1% ammonia solution. Further leach the resin with exactly 1 liter of 4% sodium sulfate collecting this effluent in a separate flask. Determine the chloride content of 100-ml. aliquots from each of the leachates by titration of the neutralized solutions with standard 0.1*N* silver nitrate using potassium chromate as the indicator. The calculation for both solutions is as follows:

$$\frac{\text{Ml.}_{AgNO_3} \times N_{AgNO_3} \times 10}{\text{sample wt.} \times \frac{\%\text{ solids}}{100}} = \frac{\text{meq. anion exchange capacity}}{\text{gram of dry Cl-form resin}}$$

The capacity calculated from the titer of the ammonia solution approximates the weakly basic capacity of the polymer and that calculated from the titer of the sodium sulfate leach the strongly basic capacity of the resin. The total anion exchange capacity is the sum of the two capacities. If only a total anion capacity is desired, the ammonia leaching may be omitted and the rinsed chloride form of the resin may be leached directly with the sodium sulfate solution. The values obtained for anion exchange capacity in this case are usually 2 to 3% lower than those obtained by the double leaching. A reproducibility of 1 part per 100 is to be expected. Unlike cation exchange resins, an accurate theoretical capacity value cannot be calculated from polymer composition.

The resin is converted to the chloride form before weighing to avoid errors due to variation in equivalent weights of various forms and to decomposition of the free-base form of certain polymers on drying. The chloride form of the resin is rinsed with alcohol instead of water to avoid hydrolysis of the salt forms of weakly basic polymers. Typical results for a series of functional groups are given in Table IV. In addition, results for materials converted to the nitrate form and hence back to the chloride form in the same manner as samples listed in Table III are given. All results are based on the weight of the chloride-form resin. The results on the quaternary resins are for four separate preparations in each case, not for four samples of a given preparation. Quaternary II is known from its titration curve to be a weaker base than quaternary I.

The nitrate form of the resin was chosen for study because it was the most common of the ions that gave incomplete regeneration in the sodium hydroxide cycle (Table III). Regeneration with chloride has been far more successful than regeneration with hydroxide. When the greatest accuracy is required and the

Table III. Effect of Resin Form on Capacity of Quaternary Resin

Resin Form	Apparent Capacity, Meq. per G.
PO_4^{---}	3.47
HCO_3^-	3.44
HSO_4^-	3.42
$C_2O_4^{--}$	3.34
CO_3^{--}	3.33
Cl^-	3.29
S^{--}	3.20
$H_2PO_4^-$	3.17
CH_3COO^-	3.08
CrO_4^{--}	2.91
Br^-	2.57
NO_3^-	2.45
$S_2O_3^{--}$	2.14
I^-	1.46
CNS^-	1.21
ClO_4^-	0.82
$Fe(CN)_6^{-4}$	0.53

Table IV. Typical Results from Anion Capacity Determinations

	Chloride Form			Nitrate Form		
	Weak base	Strong base	Total anion	Weak base	Strong base	Total anion
	Meq. per Gram					
Quaternary I						
Batch A	0.37	3.54	3.91	0.39	3.45	3.84
B	0.57	3.48	4.05			
C	0.53	3.62	4.15			
D	0.45	3.52	3.97			
Quaternary II						
Batch A	0.98	2.65	3.63	0.78	2.63	3.41
B	0.98	2.63	3.61			
C	1.09	2.52	3.61			
D	0.98	2.60	3.58			
Primary-Secondary I	4.56[a]	0.60[a]	5.16[a]	4.37	0.29	4.66
Primary-Secondary II	9.14[a]	None	9.14[a]	9.30	None	9.30
Tertiary I	3.81[a]	None	3.81[a]	3.77	None	3.77
Tertiary II	5.85[a]	None	5.85[a]			

[a] Weighed in free-base form.

resin is suspected of being exhausted with ions below nitrate, increasing the concentration of the hydrochloric acid used as a regenerant is recommended. For example, the perchlorate form of a quaternary resin has been regenerated to its original capacity by the use of 2 liters of 2*M* hydrochloric acid. Concentrations as high as 7*M* have been used with no detrimental effect on the resin, but are not recommended for routine use because of the large amount of reagent consumed per sample.

ACKNOWLEDGMENT

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Weighing Titanium(IV) Chloride for Quantitative Chemical Analysis

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A procedure has been developed for the preparation of titanium(IV) chloride for use in quantitative chemical analysis using standard serum bottles and a dry box.

THE hydrolysis of titanium(IV) chloride, when exposed to the atmosphere, has made its accurate sampling for quantitative chemical analysis difficult.

The general procedure for obtaining a sample of the tetrachloride requires the use of weighed, evacuated thin-walled glass ampoules, which must be made and prepared in the laboratory

Since a relatively large surface area of the tetrachloride is exposed in filling the ampoules, this operation and the resealing of the ampoules must be performed in a completely dry and inert atmosphere. Generally, regardless of the care exercised, some hydrolysis occurs in the ampoules. Furthermore, control of the sample size is not an easy task.

The filled ampoules are weighed, placed in a suitable acid solvent, and broken to permit the tetrachloride to be dissolved. The latter operation results in a rapid hydrolysis of the anhydrous chloride accompanied by a vigorous evolution of gases, which is difficult to control and may cause a loss of sample.

The search for a reliable sampling method resulted in the following technique, which requires materials obtainable from any supplier of chemical apparatus and a suitable "dry box" of a positive pressure type employing a dry inert gas. All operations involving the transfer of titanium(IV) chloride are performed in the dry box.

The materials required are:

Serum bottles, U. S. Army Medical Corps type, 60-ml. capacity.

Serum bottle rubber stoppers, U. S. Army Medical Corps type.

Hypodermic syringe, 5- and 10-ml.

Hypodermic needles, 22-gage stainless steel.

To facilitate the manipulation of the serum bottles and hypodermic syringes, rubber surgeon's gloves should be worn when working in the dry box.

The bottles are cleaned, dried in an oven, and cooled in a desiccator. When cool they are quickly stoppered to prevent moist air from entering them, weighed, and placed in the dry box. The titanium(IV) chloride to be analyzed (40 to 50 ml.) is stored by pouring it quickly into a dried, unweighed serum bottle, which is immediately restoppered.

Procedure. Using a 5-ml. syringe, withdraw a 0.5-ml. sample of titanium(IV) chloride for each determination to be made and inject the 0.5-ml. portions into the necessary number of weighed serum bottles. Any tetrachloride that may remain on the outer surface of the bottles or stoppers should be carefully removed with a cloth or ball of cotton moistened with *n*-heptane. The bottles should then be removed from the dry box and placed in a desiccator to bring the outer surfaces to the same condition as existed in their previous weighing. The bottles may be reweighed to determine sample weights after they have been allowed to stand in the desiccator for about 20 minutes.

Syringes used to transfer tetrachloride should be flushed with heptane and disassembled before removal from the dry box to prevent freezing of the pistons in the syringe barrels and plugging of the hypodermic needles.

Draw 8 to 9 ml. of heptane into a 10-ml. syringe and inject this into each serum bottle containing a weighed amount of tetrachloride. The hypodermic needle must not be allowed to come in contact with the tetrachloride during this addition. When all but 0.3 ml. of the heptane has been added, withdraw the needle from the bottle while keeping a slight positive pressure on the piston of the syringe to prevent any tetrachloride vapor from being trapped in the needle. Mix the tetrachloride with the heptane by swirling gently.

After the titanium tetrachloride and heptane are thoroughly mixed, slowly inject 9 to 10 ml. of 20% sulfuric acid solution, using a 10-ml. syringe and observing the same precautions as in the heptane addition. Since there is a greater increase in pressure during this addition, positive pressure must be maintained on the piston of the syringe at all times. Withdraw the needle as before and, with shaking, cool the bottle and its contents in a water bath for 1 to 2 minutes, during which time most of the tetrachloride will have dissolved in the acid. When cool, the bottles are left in the water bath until the tetrachloride is completely dissolved in the acid. This will take about 1 hour and will result in a clear liquid which will have separated into two layers.

Before removing the rubber stopper, insert a hypodermic needle through the diaphragm to release the pressure within the bottle. Then remove the stopper and transfer the contents of the bottle to a 250-ml. Erlenmeyer flask with careful and thorough washing of the stopper and bottle with distilled water. This should result in a sample volume of 80 to 90 ml.

To the sample in the Erlenmeyer flask add 50 ml. of concentrated hydrochloric acid to prevent hydrolysis. Place on a hot plate and boil gently until the heptane has evaporated, which will require approximately 5 to 10 minutes of boiling. The weighed sample of titanium(IV) chloride is now ready for analysis.

In the event that a chloride analysis is required, sulfuric acid should be used for the final addition. The possibility of a loss of hydrogen chloride in relieving the pressure increase caused by the displacement of air in the serum bottles can be avoided by the addition of a precipitant for the chloride before the pressure is released.

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Spectrophotometric Determination of Serum Copper with Biscyclohexanoneoxalyldihydrazone

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Standard copper colorimetric methods, which commonly use diethyl dithiocarbamate, give a molar absorbance index of about 8000, in aqueous solution. This low sensitivity makes impractical a reliable determination of copper on trichloroacetic filtrates of human sera. Nilsson (3) showed that the compound biscyclohexanoneoxalyldihydrazone reacts with the cupric ion in alkaline solution to give a blue color. This copper complex has a molar absorbance index of 16,000 at 600 mμ. It gives a clear, stable, blue-colored solution with constant absorbance with the cupric ion over a pH range of 7.0 to 9.0. The biscyclohexanoneoxalyldihydrazone does not give a color with any other cations or anions commonly encountered in biological materials.

THE very low concentrations of copper present in human sera require the use of very sensitive reagents for its determination. The use of diethyl dithiocarbamate or dithizone has not proved entirely satisfactory. Both of these reagents, in addition to being inadequately sensitive, lack specificity. The former reagent forms a yellow-colored colloidal suspension with microgram quantities of copper, and this complex may then be extracted with various organic solvents. However, interfering colors form with iron, cobalt, nickel, and bismuth. Also the yellow-colored complex fails to follow Beer's law over a wide range of concentrations when certain spectrophotometers or filter photometers with poor quality monochromatic light are used. Dithizone itself is colored, reacts with many metals, and in order to achieve selectivity requires complex extraction procedures in a narrow pH range. The reagent "cuproine" (2,2'-biquinoline) is specific when applied to the spectrophotometric determination of copper, but lacks the desired sensitivity (*2*). Recently, Smith has described three new copper specific reagents —2,9-dimethyl-1,10-phenanthroline (*4*), 4,7-diphenyl-1,10-phenanthroline (*5*), and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (*6*). All of these reagents are specific under the described conditions.

All of these chromogenic reagents for copper lack the desired sensitivity when applied to the determination of copper in serum. Nilsson (*3*), in a study of the condensation products of oxalhydrazide with aldehydes and ketones noted that many of the hydrazones formed gave a blue color with microgram quantities of copper salts. Nilsson found that the hydrazone formed by the reaction of 1 mole of oxalhydrazide with 2 moles of cyclohexanone gave a very intense blue color with copper. This reagent, biscyclohexanoneoxalyldihydrazone (Figure 1), was later applied to a quantitative spectrophotometric procedure for the determination of copper in paper pulp products by Wetlesen (*7*). Table I compares the sensitivity of this compound to other reagents frequently used for the determination of copper.

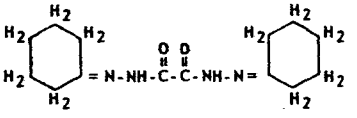

Figure 1. Biscyclohexanoneoxalyldihydrazone

This paper describes the application of this reagent to a procedure for the determination of human serum copper.

REAGENTS

Trichloroacetic Acid, 20%. Add 20 grams of reagent grade or redistilled trichloroacetic acid to redistilled water and make to volume of 100 ml.

Hydrochloric Acid, 2*N*.

Tribasic Potassium Phosphate (Merck). Make a saturated aqueous solution. The solution is hygroscopic and should be checked for complete saturation.

Phenolphthalein, 0.1% aqueous.

Biscyclohexanoneoxalyldihydrazone. Obtained from G. Frederick Smith Chemical Co. Saturated solution in 50% ethyl alcohol. Dissolve with gentle heating.

Standard Copper Solutions. Dissolve 0.3928 gram of copper sulfate pentahydrate in redistilled water and make to 1000 ml. From this stock solution prepare dilute standards containing 1 to 5 γ per ml.

The procedure used for the release of the copper from the serum proteins is an adaptation of the method described by Gubler and others (*1*).

PROCEDURE

Add 1 ml. of serum or plasma (heparinized) to a 10-ml. rounded test tube, 16 × 100 mm.

Add 0.7 ml. of 2*N* hydrochloric acid and let stand at room temperature 5 to 10 minutes.

Add 1 ml. of 20% trichloroacetic acid, mix with thin stirring rod, and centrifuge at 2500 r.p.m. for 30 minutes.

Table I. Relative Sensitivities of Various Chromogenic Reagents Used for Determination of Copper

Chromogenic Agent	Wave Length Maximum Absorption, mμ	Molar Absorbancy Index
Diethyldithiocarbamate (aqueous)	440	8,000
Diethyldithiocarbamate (amyl alcohol)	440	12,700
2,2'-Biquinoline	540	5,900
2,9-Dimethyl-1,10-phenanthroline	454	8,000
4,7-Diphenyl-1,10-phenanthroline	420	12,000
2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline	480	14,100
Biscyclohexanoneoxalyldihydrazone	600	16,000

Pipet 2.0 ml. of supernatant into graduated tube, or 19-mm. diameter Coleman cuvette, graduated to 3.5 ml.

Add one drop of phenolphthalein indicator to tubes, and mix.

Add saturated tribasic potassium phosphate dropwise with mixing (1.0 to 1.5 ml.) until solution turns just pink (pH 8.0 to 8.2).

Add 2*N* hydrochloric acid dropwise with mixing until 1 drop makes the solution colorless (1 to 3 drops usually needed). This should bring the pH of the solution within the range of 7.5 to 7.9.

Add 0.2 ml. of the biscyclohexanoneoxalyldihydrazone solution to each tube. Make to volume of 3.5 ml. with redistilled water. Mix and let stand at least 5 minutes, but read within 60 minutes.

Carry reagent blanks and standards containing 2 γ of copper through the procedure with the unknown sera. It is also advisable to carry a standard serum through with each set of unknowns.

Read at a wave length of 600 mμ in Coleman Jr. spectrophotometer (or other suitable spectrophotometer). The 3.5-ml. volume in the 19-mm. Coleman cuvette is read by placing a flat cork support approximately 8 mm. high in the bottom of the standard cuvette carrier or adapter.

EXPERIMENTAL

Sensitivity and Conformity to Beer's Law. The colored cupric biscyclohexanoneoxalyldihydrazone complex in aqueous solution

has a molar absorbance index of 16,000 at its point of maximum absorption, 600 mμ (Figure 2). This corresponds to a sensitivity of approximately 0.03 p.p.m. The cupric complex in aqueous solution conforms to Beer's law at 600 mμ in concentrations up to 4 p.p.m.

Optimum pH. Figure 3 portrays the optimum pH for color development. The optimum varies slightly with time of standing, and after 15 minutes a range of pH 7.0 to 9.0 gives a constant and maximum color, whereas if the solutions are read immediately after mixing, a slightly narrower maximum range is obtained.

Stability of Color Complex with Respect to Time and Temperature. The maximum color development is present within 5 minutes after addition of the biscyclohexanoneoxalyldihydrazone. The stability of the blue color complex is related to the concentration of phosphate buffer. However, not until 60 minutes after mixing does a gradual fading of the color begin to occur. Thereafter, this represents about 1% per hour with the concentrations of phosphate used in the procedure. With concentrations of phosphate less than one tenth of those described for use in the serum method, the blue complex in diffuse light is stable for 12 hours. At 24 hours, there is approximately 10% color fading. In the dark, the color is stable for 3 days. The color is more stable at 4° C. than at room temperature (25° C.).

Effect of Reagent Concentration. If enough reagent is present to complex all of the copper, a further increase in the concentration of the reagent will not produce a significant change in absorbance at 600 mμ. At optimum pH, experiments in which varying amounts of reagent were added to a constant amount of copper indicate that 8 moles of reagent are required to give maximum color with 1 mole of copper.

Table II. Effect of Various Anions and Cations on Cupric Biscyclohexanoneoxalyldihydrazone Color Complex

Ion	Added as	No Interference, P.P.M.	Ion	Added as	No Interference, P.P.M.
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	1000	NH_4^+	$(NH_4)_2SO_4$	1000
$Br_4O_7^{--}$	$Na_2Br_4O_7$	1000	Na^+	NaCl	1000
NO_2^-	$NaNO_2$	1000	K^+	KCl	1000
NO_3^-	$NaNO_3$	1000	Li^+	LiCl	500
SCN^-	KSCN	1000	$P_2O_7^{--}$	$Na_4P_2O_7$	500
Br^-	KBr	1000	$S_2O_3^{--}$	$Na_2S_2O_3$	100
Cl^-	NaCl	1000	CrO_4	Na_2CrO_4	50
Fl^-	NaF	1000	Ba^{++}	$Ba(C_2H_3O_2)_2.H_2O$	50
I^-	NaI	1000	Ca^{++}	$CaCl_2.2H_2O$	25
SO_3^{--}	Na_2SO_3	1000	Mg^{++}	$Mg(C_2H_3O_2)_2.4H_2O$	10
SO_4^{--}	Na_2SO_4	1000	Sn^{++}	$SnCl_2.2H_2O$	10
$C_2H_3O_2^-$	$NaC_2H_3O_2$	1000	Mn^{++}	$MnCl_2$	10
$C_6H_5O_7^{---}$	$Na_3C_6H_5O_7.2H_2O$	1000	Sr^{++}	$SrCl_2$	10
$C_2O_4^{--}$	$K_2C_2O_4.H_2O$	1000	Ce^{+++}	$Ce(SO_4)_2.8H_2O$	10
$C_4H_4O_6^{--}$	$Na_2C_4H_4O_6.2H_2O$	1000	Cd^{++}	$CdCl_2.2^1/_2H_2O$	1
CO_3^{--}	Na_2CO_3	1000	Hg^+	$HgNO_3.H_2O$	1
HCO_3^-	$NaHCO_3$	1000	Hg^{++}	$Hg(NO_3)_2.H_2O$	1
MoO_4^{--}	$Na_2MoO_4.2H_2O$	1000	Cr^{+++}	$CrCl_3$	1
Wo_4^{---}	$Na_2Wo_4.2H_2O$	1000	Co^{++}	$Co(C_2H_3O_2)_2.4H_2O$	1
AsO_4	$NaHAsO_4.7H_2O$	1000	Fe^{+++}	$FeCl_3$	1
PO_4^{---}	$Na_2PO_4.12H_2O$	1000	Al^{+++}	$AlCl_3$	1
Ag^+	$AgNO_3$	1000	Versene	Ca Versenate	0.5

Effect of Presence of Foreign Ions. The method employed for examining these effects was the same as in the outlined procedure, except that the foreign ion was added in solution to a small volume of 2*N* hydrochloric acid containing microgram quantities of copper. If a precipitate formed after color development, it was removed by centrifuging. Table II lists the effects of some of the more common ions. An interference is defined as an alteration of more than ±2% in the absorbance of an aqueous solution at pH 7.0 to 9.0 containing 1 p.p.m. of cupric ion.

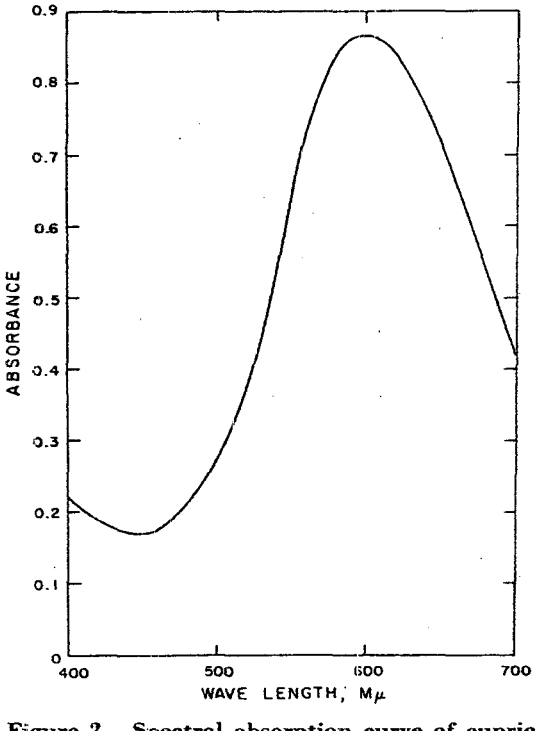

Figure 2. Spectral absorption curve of cupric biscyclohexanoneoxalyldihydrazone at pH 7.5

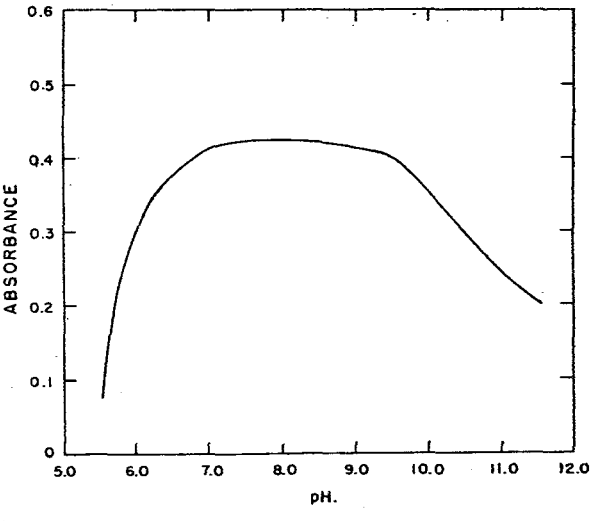

Figure 3. Curve of absorbance of cupric biscyclohexanoneoxalyldihydrazone complex versus pH at 600 mμ

Evaluation of concentrations above 1000 p.p.m. were made in only a few instances. Many of the cations interfered at a low concentration because of the turbidity developed at the pH 7.5 used for color development. Lead, zinc, and nickel interfered at concentrations of 0.5 p.p.m. with formation of a precipitate. Cyanide prevents color development at concentrations of less than 0.1 p.p.m. All of the interferences noted were of a negative character, and none of the 48 ions tested gave any color with this reagent. With certain anions, color development was accelerated, and with some cations full color development was delayed. Many of the cations that interfere by formation of a precipitate

Table III. Recovery and Precision of Copper Added to Serum, Expressed in Absorbance

1 Ml. of Serum	1 γ of Cu	1 Ml. of Serum + 1 γ of Cu: Found	Calculated	Deviation, γ
0.088	0.072	0.162	0.160	+0.03
0.089	0.072	0.162	0.161	+0.01
0.086	0.072	0.163	0.158	+0.06
0.091	0.070	0.160	0.161	−0.02
0.089	0.071	0.160	0.160	0

of the metal hydroxides or hydrated oxides at alkaline pH can be prevented by addition of citrate. At 3000 p.p.m. citrate does not interfere with color, but at 6000 p.p.m. an inhibition of color occurs. Iron may be present in concentrations up to more than 10 p.p.m. if citrate is present.

Recovery and Precision. Table III lists the recovery and degree of precision obtained when copper is added to normal pooled serum in the range of concentrations commonly found in serum. Presumably this method measures total serum copper, since comparable results are obtained with this reagent when applied both to trichloroacetic acid filtrates of sera and wet ashed digests of sera. Also, when a trace of radioactive copper-64 was added to human serum and the specific activity of the copper in the trichloroacetic acid filtrate compared with the specific activity of the copper in a wet ash digest, identical values were obtained.

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A Colorimetric Coulometer

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A new type of coulometer has been investigated, operating on the principle of the measurement of a color change produced by the electrode reaction. The most sensitive coulometer was obtained by having the electrode reaction change the pH of the solution and measuring this pH with an acid-base indicator. This coulometer could be used in the range 0.01 to 1.00 coulomb. Other electrode reactions described could be used for measurements up to 10 coulombs.

IN THE coulometric investigation of processes at small solid electrodes it was necessary to use a coulometer that could measure one coulomb and less. The common coulometers, such as the silver (*3*) and oxyhydrogen coulometer (*2*), require a high degree of precision in the measurement of quantities of electricity this small. Most of the electrical devices (*1*) were prohibitive in cost. Therefore, the possibility of using a simple laboratory colorimeter as a coulometer was investigated. The colorimeter could measure the number of coulombs by measuring the change in color in a solution caused by an electrode reaction in the solution.

Any electrode reaction which could of itself produce or destroy a color, or a reaction, which produced products, which in turn, could cause some type of indicator to change color, could be used in this colorimetric coulometer.

EQUIPMENT AND PROCEDURE

The coulometer was designed around a 12-mm. sample tube of a clinical-type Klett-Summerson colorimeter. To prevent the anode and cathode reactions from interfering with each other the two half cells were separated by a salt bridge. A rotating electrode and stirrer were combined for the coulometer half cell as shown in Figure 1. Rotary motion to the electric stirrer was supplied by an inverted magnetic stirrer. This type of drive was used to aid in removal of the electrode, since, before each colorimeter reading was taken, the electrode-stirrer was removed to prevent any error from absorption and reflection of light by the wire.

In the investigation of the various reactions, the number of coulombs was measured by timing the passage of a constant current through a galvanometer shunt system which had been previously calibrated as a microammeter. The constant current source was obtained by operating a 45-volt battery into a heavy load.

RESULTS

Three classes of color producing reactions were investigated—a reaction in which a colored substance is removed or produced by direct reaction at the electrode; a reaction in which the solute reacts at the electrode and the products of this reaction subse-

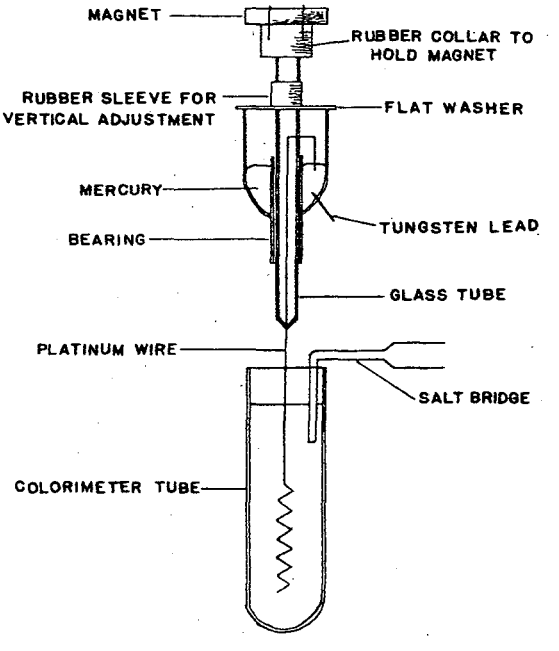

Figure 1. Coulometer half cell

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quently react with some indicator; and a reaction in which the solvent reacts at the electrode and the products of this reaction subsequently react with some indicator.

Several oxidation-reduction indicators were investigated as examples of the direct reduction or oxidation of a colored substance. These indicators did not give good results because they tended to precipitate on the electrode. Potassium permanganate was reduced with 100% current efficiency, and was suitable for use as a coulometer in the range 0.1 to 3.0 coulombs.

Several reactions involving the oxidation or reduction of a solute, and its subsequent reaction with an indicator, were tried. The best of these was the formation of the copper–triethanolamine complex. Both the dissolution and deposition of copper were studied, and the formation of the blue copper complex in a 28% triethanolamine solution was the most satisfactory. There was a linear relationship between the colorimeter reading and the number of coulombs passed, as shown in Figure 2. By comparing the colorimeter readings with known copper standards, the current efficiency of the reduction was found to be nearly 100%. The low color intensity of this copper complex limited the sensitivity of this coulometer to about 1 to 10 coulombs.

The reaction of the solvent at the electrode and the subsequent reaction of these products with an indicator proved to be the most sensitive of the reactions investigated. The solvent, water, reacted at the cathode causing an increase in pH and at the anode causing a decrease in pH. This change in pH was shown colorimetrically by the action of acid base indicators.

Thymol blue, neutral red, alizarin yellow R, and *o*-cresol red were some of the indicators studied. Typical acid-base neutralization curves were obtained with these indicators. Figure 3 shows the curves obtained with *o*-cresol red.

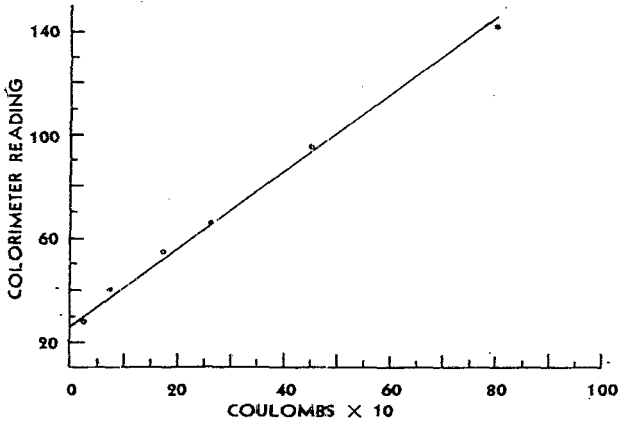

Figure 2. Dissolution of copper to triethanolamine complex

Red filter. Transmittance units of 640 to 700 mμ

The equation of the curve can be derived from theoretical considerations. With the proper choice of filter, the colorimeter can be made sensitive to only one form of the indicator. For example, with thymol blue in the cathode compartment the color changes from the yellow acid form to the blue base form, which corresponds to the undissociated molecule and the anion, respectively. Using a red filter (transmittance limits of 640 to 700 mμ) the colorimeter will be insensitive to the concentration of the yellow undissociated molecule, and therefore, as the concentration of the anion increased by the passage of current in the half cell, direct measurement of the anion is obtained by the colorimeter. The concentration of the anion of the indicator is essentially zero initially whereas at the end of the neutralization curve, the concentration of the anion is equivalent to the initial concentration of the dye. In the cathode compartment, the

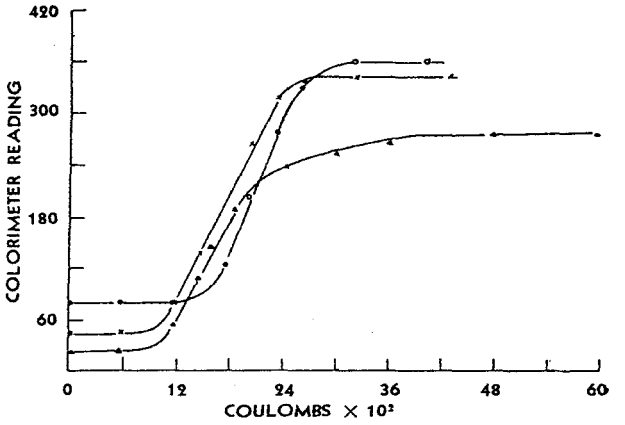

Figure 3. *o*-Cresol red acid-base indicator

Alcohol concentration, 10% by volume
Green filter. Transmittance limits of 520 to 580 mμ
●. 1.66×10^{-4} mole per liter
×. 0.830×10^{-4} mole per liter
▲. 0.415×10^{-4} mole per liter

hydrogen ion concentration can be expressed as $[H^+] = [OH^-] + [In^-]$ − faradays passed, where $[In^-]$ is the concentration of the anion. Since the $[In^-]$ is proportional to the colorimeter reading, then $[In^-] = k \times R$, where k is an instrument constant, and R the reading of the colorimeter. Substituting this proportionality into the above equation, then

$$\text{Faradays passed} = k \times R + [OH^-] - [H^+] \qquad (1)$$

Inserting the equations for the equilibrium of an acid-base indicator, $K_{HIn} = [H^+][In^-]/[HIn]$, and for water, $[OH^-] = K_w/[H^+]$, Equation 1 becomes

$$\text{Faradays passed} = k \times R \frac{K_w \times [In^-]}{K_{HIn} \times [HIn]} - K_{HIn} \frac{[HIn]}{[In^-]} \qquad (2)$$

Since the $[HIn] = [In^-]_{final} - [In^-]$

Equation 2 is

$$\text{Faradays passed} = k \times R \frac{K_w \times R}{K_{HIn}(R_{final} - R)} - \frac{K_{HIn}(R_{final} - R)}{R} \qquad (3)$$

Figure 4 shows the agreement between the theoretical and experimental curve for thymol blue. Although the agreement is

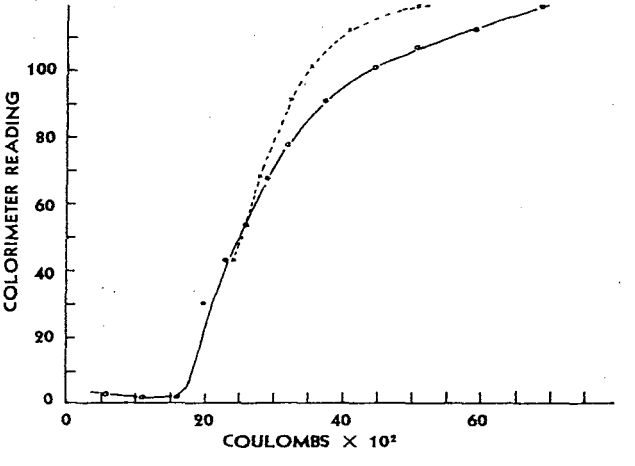

Figure 4. Thymol blue acid-base indicator

Alcohol concentration, 10% by volume
Red filter. Transmittance limits of 640 to 700 mμ
●. Experimental results
×. Theoretical results

reasonable, the coulometer must be standardized for practical use. Similar equations can be derived for the more complex situation when both the acid and base forms absorb. However, the agreement between theoretical and practical curves is not so good.

In practice a solution of the indicator is prepared in a concentration of 0.1 to 1.0 $\times 10^{-4}$ mole per liter, depending on the color intensity of the indicator. A standardization curve is plotted. Then one is prepared to use the coulometer. For the greatest sensitivity it is best to pass current until the readings are on the steep straight-line portion of the graph. The reproducibility of this portion of the curve is within the accuracy of the colorimeter. This coulometer is most useful in the range of 0.01 to 1.00 coulomb.

These colorimetric coulometers with the wide variety of reactions available offer possibilities for use in numerous cases where small quantities of electricity are to be measured.

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Determination of Small Amounts of Chlorate in Ammonium Perchlorate

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A colorimetric microdetermination of chlorate in ammonium perchlorate is based on the production of a colored substance from brucine with chloric acid.

WHEN ammonium perchlorate is to be used in explosive or pyrotechnic compositions, it must be practically free of chlorate, for chlorate lowers the temperature of deflagration of ammonium perchlorate considerably. According to the standard texts, the chlorate concentration should not exceed 0.02% (*4*). The methods for the determination of chlorate (*1*), which are based on the reduction of the chlorate to chloride, are cumbersome. The method recommended by Lunge (*3*)—i.e., reduction of the chlorate with ferrous sulfate and titration of the ferrous ion in excess with potassium permanganate—did not give consistent results. The colorimetric methods suggested (*5*) did not seem to be reliable. Therefore, an adaptation of the color reaction with brucine (*6, 7*) was worked out for use in quantitative analysis.

EXPERIMENTAL

Reagents. Brucine, Revector indicator preparation (Hopkins and Williams). The 5% (weight by volume) solution of the reagent in glacial acetic acid (AnalaR) was kept in a glass-stoppered bottle.

Sulfuric acid, 30 to 32%, is prepared by dilution of 80 ml. of sulfuric acid (AnalaR, density 1.84) with distilled water to 250 ml. The purity of the sulfuric acid is of prime importance. Acid with a dark tint gives consistently low results.

Potassium chlorate (May and Baker) was recrystallized once from water and dried.

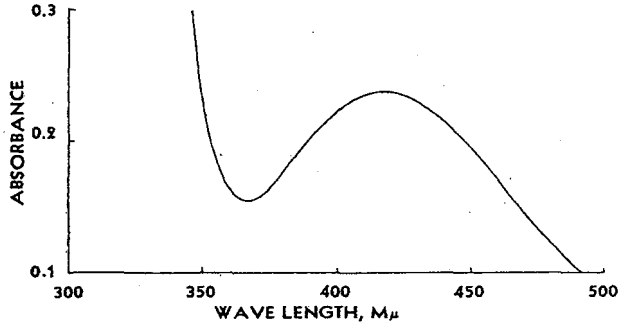

Figure 1. Absorption spectrum of colored compound

Five per cent brucine solution, 0.5 mg., in glacial acetic acid, containing 0.008% of potassium chlorate

Ammonium perchlorate was prepared by neutralization of technical perchloric acid with ammonium hydroxide solution and recrystallized four times from water. It was completely free from chlorate, as indicated by the negative spot test with brucine (*2*).

The standard solution of ammonium perchlorate contained an amount of potassium chlorate equivalent to 0.02% of ammonium chlorate. A quantity of 120.8 mg. of potassium chlorate was dissolved in 100 ml. of distilled water. Five grams of ammonium perchlorate (solubility 10.74 grams per ml. of water at 0° C.) were weighed into a 50-ml. volumetric flask and were dissolved in the required amount of distilled water. Then this solution, to which 1 ml. of the potassium chlorate solution was added, was made up to volume with water. This solution is equivalent to 0.02 weight % of ammonium chlorate in ammonium perchlorate.

Apparatus. A Hilger Spekker absorptiometer H760 was used, fitted with No. 1 Kodak filters (transmittance at 405 to 455 mμ) and 2-cm. Corex absorption cells. The filter was chosen in accord with the absorption spectrum of the color produced in the reaction (see Figure 1). The principal spectral band lies at 435 mμ. The absorption spectra were measured with a Beckman DU quartz spectrophotometer, using 1-cm. quartz cells.

Procedure. PREPARATION OF CALIBRATION CURVE. From an automatic 5-ml. microburet, fitted with a long capillary tip which reached to the center of the volumetric flasks, 0.5, 1, 2, 3, 4, and 5 ml., respectively, of the standard solution were measured into six 25-ml. volumetric flasks and 5 ml. of distilled water into a seventh one (this gave the same blank as ammonium perchlorate solution). Then 10 ml. of ice-cold sulfuric acid was added to each flask, and the neck of the flask was rinsed with the acid to remove traces of adhering substance; then the flasks were shaken thoroughly. Finally, 0.5 ml. of the brucine solution was added and the content of the flasks was mixed again. The flasks were immersed in a boiling water bath for 15 minutes, cooled with ice, and filled up to the mark with distilled water. The color of the solutions was then measured against that of the blank, and the absorbance ($D = \log I_0/I$) plotted against weight per cent of ammonium chlorate in ammonium perchlorate. A straight line was obtained from the observed points of 0.01, 0.02, 0.04, 0.06, and 0.08% of ammonium chlorate, respectively, absorbance 0.0448, 0.0880, 0.1798, 0.2750, and 0.3624. For best results, measurements should be made immediately after the formation of the colored product is completed. The intensity of the color remains constant at a temperature of 10° C. for 2.5 hours. After 20 hours, a 6.5% decrease of the absorbance has been noted.

ANALYSIS OF UNKNOWN SAMPLES. A quantity of 10.00 grams of the ammonium perchlorate sample was weighed into a 100-ml. volumetric flask, dissolved in distilled water, and made up to volume. If the sample contains insoluble impurities, the solution must be filtered. This solution, 1 to 5 ml. (depending on the chlorate content), is measured into a 25-ml. volumetric flask and the analysis is carried out as described. Using the calibration curve, the chlorate content is determined from the absorbance measured.

RESULTS

Some of the results obtained are summarized in the following tables.

Table I. Absorbance Differences (against Blank Value) as Function of Ammonium Chlorate Content[a]

% Ammonium Chlorate in Ammonium Perchlorate	Δ Absorbance × 10³				
	1	2	3	4	5
0.01	045	047	045	045	042
0.02	088	088	089	087	088
0.04	180	181	179	179	180
0.06	275	275	278	269	273
0.08	359	363	361	368	361
0.10	430	434	435	...	445

[a] Five determinations.

Table II. Error in Chlorate Determination

No. of Detn.	% Ammonium Chlorate Added[a]	% Ammonium Chlorate Found	Mean Difference
9	0.01	0.0096 ± 0.00025	−0.0004
15	0.02	0.0194 ± 0.00019	−0.0006
15	0.04	0.0398 ± 0.00025	−0.0002
10	0.06	0.0606 ± 0.00028	0.0006
10	0.08	0.08 ± 0.00033	0
9	0.10	0.096 ± 0.00148	−0.004

[a] Calculated from potassium chlorate added.

The method gives excellent results below the 0.1% concentration of ammonium chlorate. For concentrations greater than 0.1%, the results tend to become somewhat low.

Regarding the possible interference of foreign ions, systematic experiments were carried out with chloride and ferric ions. As much as 0.1% of ammonium chloride in the ammonium perchlorate had no effect. The ferric ion did not interfere up to a quantity of 0.005%; larger amounts gave too high absorbance readings. Samples containing large quantities of iron ion should be treated with ammonia solution and then filtered to remove the iron ion. Periodate and nitrate interfere with this method. However, iodate gave no measurable color with the reagent below the 0.033 weight % concentration in the ammonium perchlorate. These ions are not likely to be present in samples of ammonium perchlorate.

Heating of the solution from 15 to 30 minutes had no adverse effect on the accuracy of the analysis.

DISCUSSION

Comparison of the absorption spectra of brucine (Figure 2) and the colored compound (Figure 1) leads to the hypothesis that the ring system of the former is oxidized by the chloric acid. It is most likely (supported by unpublished results on the determination of nitroglycerin with brucine), that all oxidizing agents convert brucine into the same compound or at least compounds characterized by the same chromophoric groups.

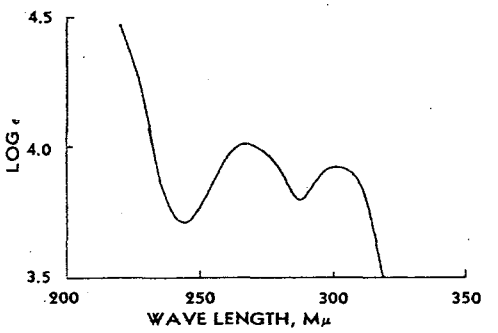

Figure 2. Absorption spectrum of brucine

Brucine, 5.1 mg., in 1 ml. in glacial acetic acid, diluted successively with 30% sulfuric acid

ACKNOWLEDGMENT

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Spectrophotometric Determination of Nickel in Tungsten Powder

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A rapid and accurate method has been developed for the determination of microgram quantities of nickel in tungsten powder. With this method the nickel is separated from all interfering elements by a chloroform extraction of the bivalent nickel complex of dimethylglyoxime in the presence of the hydrogen peroxide used to dissolve the metal powder. The determination is made by spectrophotometric measurement of the diethyldithiocarbamate complex of nickel. Nickel, 1 to 50 γ, can be determined in tungsten powder with an average deviation of less than 1 γ.

NICKEL if present within the crystal structure of tungsten powder or as a dope will alter its physical properties. As an impurity, its accurate determination is usually lengthy and it requires a preliminary separation before a suitable colorimetric method can be utilized for the small amounts present.

Fettweis (*3*) has attempted to determine nickel in tungsten steels by precipitating with dimethylglyoxime, but found that the nickel precipitate of dimethylglyoxime retains some tungstic oxide and that results are from 0.05 to 0.1% high. However, no previous methods for the determination of small amounts of nickel in tungsten powder have been reported.

Dimethylglyoxime has been mentioned frequently in the literature as a reagent forming a soluble, colored complex with nickel, either complexing with quadrivalent nickel, or if oxidized complexing with bivalent nickel. This reagent has long been used for the detection of nickel (*2*). Bivalent nickel can be separated by a chloroform extraction of the insoluble, bivalent nickel complex of dimethylglyoxime (*6*). Oxidizing agents such as nitrates, ferricyanides, peroxides, and permanganates have been reported to prevent the formation of this insoluble nickel pre-

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cipitate (*7*); however, reliable results have been obtained in the mixtures employed in the determination of nickel in steel.

In this work the bivalent nickel complex of dimethylglyoxime has been satisfactorily extracted with chloroform in the presence of an excess of hydrogen peroxide. The method employed had been first used by Alexander, Godar, and Linde (*1*), who used two reactions of nickel together, a separation of nickel dimethylglyoxime with chloroform and the extraction of a diethyldithiocarbamate complex with isoamyl alcohol, the nickel being determined by spectrophotometric measurement of the yellow-green complex.

APPARATUS AND REAGENTS

Dimethylglyoxime, 0.1%. Dissolve 0.25 gram of reagent grade dimethylglyoxime in 50 ml. of absolute alcohol and dilute to 250 ml. with distilled water.

Ammonium Citrate (dimethylglyoxime, purified), 20%. Dissolve 200 grams of ammonium citrate in 600 ml. of distilled water, adjust with ammonium hydroxide to pH 9.0 to 9.5, and transfer to a separatory funnel. Add 10 ml. of dimethylglyoxime reagent and extract three times with 30-ml. portions of chloroform. Filter the aqueous layer and dilute to 1 liter with distilled water.

Sodium Diethyldithiocarbamate, 0.2%. Dissolve 1 gram of sodium diethyldithiocarbamate in 100 ml. of distilled water, filter, and dilute to 500 ml. with distilled water.

Ammonium Citrate (sodium diethyldithiocarbamate, purified), 20%. Dissolve 200 grams of ammonium citrate in 600 ml. of distilled water, adjust with ammonium hydroxide to pH 9.0 to 9.5, and transfer to a separatory funnel. Add 10 ml. of sodium diethyldithiocarbamate reagent and extract with 20-ml. portions of carbon tetrachloride until the organic layer is colorless. Add 5 ml. of carbamate reagent and again extract with carbon tetrachloride. If the organic layer remains yellow, add more carbamate reagent and continue the extractions until the layer is colorless.

Hydrochloric Acid, 0.5*N*. Dilute 40 ml. of concentrated hydrochloric acid to 1 liter with distilled water.

Standard Nickel Stock Solution. Dissolve 0.500 gram of pure nickel in 20 ml. of (1 + 1) nitric acid and dilute to 1 liter with distilled water.

Standard Nickel Working Solution (1 ml. = 5 γ of Ni). Dilute 10 ml. of the standard nickel stock solution to 1 liter with 0.5*N* hydrochloric acid.

All transmittance measurements were made with a Beckman Model B spectrophotometer in combination with a blue-sensitive phototube and a Beckman No. 12216 filter, using 1.000-cm. Corex cells. The pH measurements were made with a Beckman Model M pH meter.

PREPARATION OF CALIBRATION CURVE

Add 0, 1, 2, 3, 4, and 5 ml. of standard nickel working solution to 125-ml. separatory funnels and dilute to exactly 25 ml. with 0.5*N* hydrochloric acid using a 25-ml. graduate. To these solutions add 5 ml. of ammonium citrate solution (carbamate, purified), a small piece of red litmus paper, and sufficient ammonium hydroxide to make slightly alkaline plus an excess of 8 drops. Add exactly 10.0 ml. of isoamyl alcohol and 5 ml. of carbamate reagent; stopper and shake vigorously for 2 minutes. Allow the layers to separate completely, discard the aqueous layer, and drain the organic layer into a clean, dry spectrophotometer cell through a small piece of cotton placed in the tip of the stem of the separatory funnel. Determine the transmittance at 385 mμ using the combination of a blue-sensitive phototube and a Beckman No. 12216 filter. Distilled water is used in the reference cell.

PROCEDURE

Weigh a sample of tungsten powder containing preferably 10 to 50 γ of nickel into a 100-ml. beaker. Carry through a reagent blank, following the same procedure and using the same amount of reagents used for treatment of the sample. Read reagent blank against the reference cell containing water and subtract from the micrograms of nickel found in the sample or aliquot portion. Wash down the sides of the beaker with a few milliliters of distilled water, and add 5 ml. of 30% hydrogen peroxide; cover with a watch glass and allow to stand until the vigorous reaction has subsided. With distilled water wash down the sides of the beaker and the watch glass, and place on a hot plate until the solution begins to boil or until the metal powder has completely dissolved. Cool to room temperature, and add 10 ml. of ammonium citrate solution (dimethylglyoxime, purified). If the nickel content is too high for direct treatment, dissolve an appropriately sized sample in 10 ml. of hydrogen peroxide, dilute to 50 ml. and take an aliquot portion, and continue by adding 10 ml. of ammonium citrate solution. Dilute to approximately 40 ml. with distilled water; add solid hydroxylamine hydrochloride until the solution is definitely acidic, and finally add a small excess. A large excess of hydroxylamine hydrochloride will neither interfere nor contribute to the blank, the excess only increasing the amount of ammonium hydroxide needed for subsequent neutralization. (Only one lot of hydroxylamine hydrochloride was used in this work; however, another lot supplied by a different manufacturer showed a different odor, smaller crystal size, lower acidity, and lower reducing power. Later work made use of this less acid reagent and made it necessary to add hydroxylamine hydrochloride before adding the citrate buffer to effect complete reduction, rather than after the buffer. The change in order of adding these two reagents produced the same effective reduction and desired results as those experienced with the more acid hydroxylamine hydrochloride.)

Using a pH meter adjust the acidity of the solution to pH 8.5 by adding ammonium hydroxide. Cool the solution to room temperature, transfer to a 125-ml. separatory funnel, and add 5 ml. of dimethylglyoxime reagent. Upon adding this reagent carefully note whether the colorless solution changes to an orange-red color; if a definite coloring of the solution is noted, oxidation has taken place. In any case continue by adding 10 ml. of chloroform, stopper, and shake vigorously for 1 minute. Allow the layers to separate, and drain the chloroform layer into a second 125-ml. separatory funnel containing 25 ml. of (1 + 50) ammonium hydroxide. Add 5 ml. of chloroform and repeat the extraction, draining this into the second funnel. If the aqueous layer is now colored, or coloration has been previously noted, indicating oxidation has taken place, drain the layer into the original beaker. To this aqueous solution, add an excess of hydroxylamine hydrochloride, adjust to pH 8.5 with ammonium hydroxide, transfer back into the first separatory funnel, and extract with an additional 5-ml. portion of chloroform. Combine this extract with the previous extracts; this last extraction is not necessary if there is no evidence of coloration. Shake the combined extracts for 1 minute and allow the layers to separate prior to draining the chloroform layer into a small, dry beaker. Add a few milliliters of chloroform to the remaining dilute ammonium hydroxide, shake, allow the layers to separate, and drain the chloroform layer into the previous chloroform extract. Discard the aqueous layer, washing out the separatory funnel with distilled water. Transfer the chloroform extracts in the beaker back into the separatory funnel, carefully rinsing out the beaker with a few milliliters of chloroform which is added to the separatory funnel. Add 25 ml. of 0.5*N* hydrochloric acid, shake vigorously for 1 minute, allow the layers to separate completely, and discard the organic layer. Add a few milliliters of carbon tetrachloride, shake for a moment, and drain off the organic layer as completely as possible. The remaining aqueous layer is treated as in the preparation of the calibration curve beginning with the addition of the citrate buffer solution.

DISCUSSION OF METHOD

Dissolution of tungsten powder with hydrogen peroxide seems to be the most effective and rapid method available but previous reports seem to indicate its presence would prevent the complete precipitation of nickel dimethylglyoxime and cause the formation of a soluble, orange-red complex which would subsequently be insoluble in the chloroform extract.

If the reported interference of hydrogen peroxide could be eliminated, after separation with dimethylglyoxime and chloroform, the nickel could be converted to the diethyldithiocarbamate complex and extracted with isoamyl alcohol. The literature shows that metals giving colored precipitates with diethyldithiocarbamate, soluble in organic solvents, are nickel, copper, bismuth, and iron (*4*). The use of ammoniacal citrate solutions prevents the reaction with iron, and the nickel is separated completely from other potential interfering elements, such as cobalt and bismuth, by the dimethylglyoxime extraction (*1*). High concentrations of copper are partially extracted with the dimethylglyoxime, but may easily be removed by an intermediate wash with dilute ammonium hydroxide. Maximum absorbance for the nickel diethyldithiocarbamate complex occurs at 385 mμ at the very edge of the visible spectrum. The calibration curve, Figure 1, very nearly approaches a straight line. This slight curvature is said to be due to the failure of the optical systems of certain spectrophotometers to produce light beams that are spec-

trally pure rather than failure of the measured color to obey Beer's law (*1*).

The experiments below showed nickel could be satisfactorily extracted as the bivalent nickel complex of dimethylglyoxime with chloroform in the presence of as much as 5 ml. of 30% hydrogen peroxide. Standard solutions of nickel, to which 5 ml. of 30% hydrogen peroxide was added, were treated as unknowns and carried through the entire procedure. Satisfactory recoveries were obtained as evidenced in Table I with an average deviation of nickel recovered from the nickel added of 0.4 γ; there was never any evidence of oxidation, indicated by a colored, aqueous layer during the dimethylglyoxime separation, when standard nickel solutions were put through the procedure. If partial precipitation of nickel dimethylglyoxime in the presence of hydrogen peroxide is due to the formation of quadrivalent nickel, the ammonium citrate, which is present during the determination to keep iron in solution, may act as a reducing agent and counteract the effect of the hydrogen peroxide (*8*).

However, in the presence of a tungsten powder, designated here as sample A, and hydrogen peroxide, incomplete recovery of known amounts of nickel is evidenced in Table II. Sandell (*5*) has said manganese in the oxidized state has a tendency to oxidize the nickel present, making the addition of hydroxylamine hydrochloride necessary to reduce any manganese present. Such may be the case in the presence of tungsten, one possible explanation being that dissolution of tungsten powder forms soluble peroxytungstic acid, which may in turn oxidize the nickel. To counteract this effect solid hydroxylamine hydrochloride was added until the solution was definitely acid. When treated in the prescribed manner satisfactory results were obtained. The effect of hydroxylamine hydrochloride on a tungsten powder A and on metal powder A to which known amounts of nickel were added, in Table III, can be compared to values in Table II using the same sample of metal powder but with no hydroxylamine hydrochloride added. The average deviation of nickel recovered from the nickel present is 1 γ.

To test the procedure further, tungsten powder samples of high nickel content, samples B, C, D, and E, were analyzed, with results as shown in Table IV. These samples were dissolved in 10 ml. of 30% hydrogen peroxide, diluted to 50 ml. with distilled water, and appropriately sized aliquots were treated as before.

Table I. Effect of Hydrogen Peroxide on Standard Nickel Solutions

	Micrograms of Nickel		
Detn.	Added	Recovered	Deviation
1	24.9	25.5	+0.6
2	24.9	24.5	−0.4
3	24.9	24.6	−0.3
		Av. dev.	0.4

Table II. Effect of Hydrogen Peroxide in Presence of Tungsten

Tungsten Powder A Taken, G.	Micrograms of Nickel	
	Added	Recovered
1.00	None	3.6
1.00	24.9	25.0
1.00	10.0	10.5
0.500	None	1.5

Table III. Effect of Hydroxylamine Hydrochloride in Presence of Hydrogen Peroxide and Tungsten

Tungsten Powder A Taken, G.	Micrograms of Nickel				% Ni in Metal Powder
	Added	Total present	Total recovered	Dev.	
1.00	None	..	17.5	...	0.00175
1.00	24.9	..	41.5	...	0.00166
				Av. % Ni	0.00171
0.7013	10.0	21.9	21.7	−0.2	0.00167
0.7079	24.9	37.0	35.3	−1.7	0.00147
0.5026	24.9	33.5	32.5	−1.0	0.00151
			Av. dev.	1.0	

Table IV. Analysis of Tungsten Powders of High Nickel Content

Sample	Gram Sample	Aliquot Portion	% Ni	Av.
B	0.4499	0.2	0.0155	0.0159
	0.4499	0.1	0.0162	
C	0.5255	0.2	0.0532	0.0536
	0.5255	0.1	0.0550	
	0.4308	0.2	0.0537	
	0.4308	0.1	0.0563	
	0.4308	0.1	0.0544	
	0.3649	0.2	0.0513	
	0.3649	0.1	0.0512	
D	0.3188	0.2	0.0416	0.0417
	0.3188	0.1	0.0417	
E	0.4119	0.2	0.0159	0.0169
	0.4119	0.1	0.0178	

Table V. Blends of High and Low Nickel Content Samples

Gram of Sample A	Gram High Nickel Content Samples	Aliquot Portion	Total Ni		
			Present	Recovered	Dev.
0.3052	0.1032 (B)	Total	21.6	20.8	−0.8
0.3037	0.2154 (C)	0.2	24.1	23.5	−0.6
0.3041	0.1165 (D)	0.2	10.8	12.0	+1.2
0.3132	0.1473 (E)	Total	30.2	29.8	−0.4
				Av. dev.	0.8

In Table V are shown the results of blending metal powders with high and low nickel content. The total nickel recovered in each case is compared to the total nickel present calculated from the average percentages of high and low nickel content samples from Tables III and IV. The average deviation of nickel recovered from the nickel present is 0.8 γ.

A reagent blank was run along with each set of determinations, the average blank being approximately 5 γ of nickel. Distilled water was used the majority of the time, since distilled water passed through a demineralizer column did not appreciably reduce the reagent blank.

SUMMARY

Microgram amounts of nickel may be determined in tungsten powder with satisfactory precision and accuracy by a preliminary separation of nickel with dimethylglyoxime and the subsequent spectrophotometric measurement of the developed diethyldithiocarbamate complex. However, the addition of hydroxylamine hydrochloride is necessary in the presence of hydrogen peroxide and tungsten to effect complete recovery of nickel. One explan-

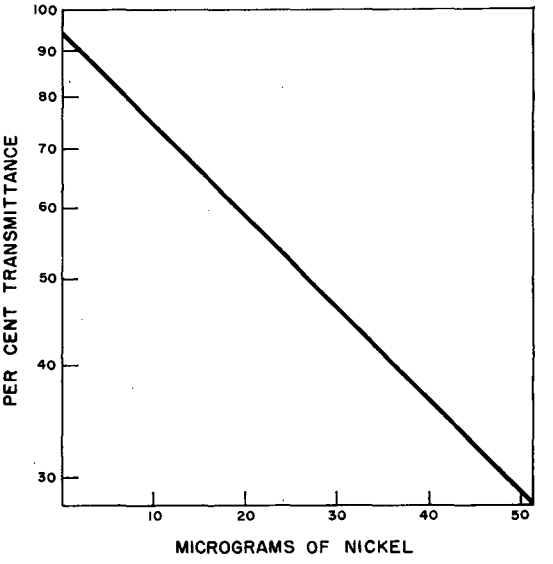

Figure 1. Calibration curve for determination of nickel

ation is that when tungsten powder is dissolved in hydrogen peroxide a peroxy acid of tungsten is formed which, in turn, oxidizes the nickel to the quadrivalent state, a complex that is not extracted in the dimethylglyoxime separation. Although standard samples were not available, various synthetics of both high and low nickel content samples were made in order to ascertain both the accuracy and precision of the method.

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CRYSTALLOGRAPHIC DATA

96. D-Fructose 2,4-Dinitrophenylhydrazone Pyridine Solvate

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THE preparation of D-fructose 2,4-dinitrophenylhydrazone pyridine solvate ($C_{12}H_{16}N_4O_9.C_5H_5N$) has been described by White and Secor (*1*). Some of their yellow crystalline preparations were used in this study.

It was necessary to use fresh material when determining refractive indices by immersion in order to avoid trouble from a thin film of decomposed material which developed on the crystals after aging. Old crystals could be used if they were broken to expose fresh surfaces at the time of immersion.

A crystal mounted on a goniometer head was used for x-ray measurements, optical goniometry, and microscopical observations. The faces of the crystals were always striated, usually giving poor and multiple signals on the optical goniometer. Twinning also caused trouble, but the average of several sets of measurements on several crystals was used for the values given here (Figure 1). The angles calculated from x-ray data agree well with the optical goniometric values.

CRYSTAL MORPHOLOGY.

Crystal System. Monoclinic, Class 4 (only a 180° axis of symmetry).

Form and Habit. Figure 2 shows the prismatic blades and needles formed on a slide by crystallization from pyridine. The elongation is parallel to *b*. The blades usually lie on {100} and show an obtuse end angle. Except for {100} the forms shown in Figure 2 are inconspicuous on most crystals. Loose sheaflike clusters of needles are common. The optical activity of this compound (*1*) requires the absence of a plane of symmetry but as ordinarily developed (Figure 2) they appear to have one.

Interfacial Angles. $100 \wedge 403 = 120°$ (120° 12′ x-ray), $403 \wedge \bar{1}01 = 126°$ (126° 13′ x-ray), $100 \wedge 10\bar{1} = 114°$ (113° 35′ x-ray).

Twinning. End views of mounted crystals usually show from two to several lamellae, which appear parallel to (100); however, x-ray evidence shows that the twins share the *a* and *b* axes rather than *c* and *b* and that the twinning plane is perpendicular to *a*. The striations or steps on (100) prevent certain recognition of the small angle (1° 15′) that should exist between the twin line and (100).

X-RAY DIFFRACTION DATA

Space Group. $C_2^2 - P2_1$ (only first 6 orders of *b* axis observed).

Cell Dimensions. $a = 20.133$ A., $b = 5.746$ A., $c = 8.788$ A.

Axial Ratio. $a:b:c = 3.504:1:1.529$.

Beta Angle = 91° 15′. The choice of axis *a* is based on the diffraction pattern (Weissenberg). There is no crystal face containing axis *a*, although it is perpendicular to the twinning plane.

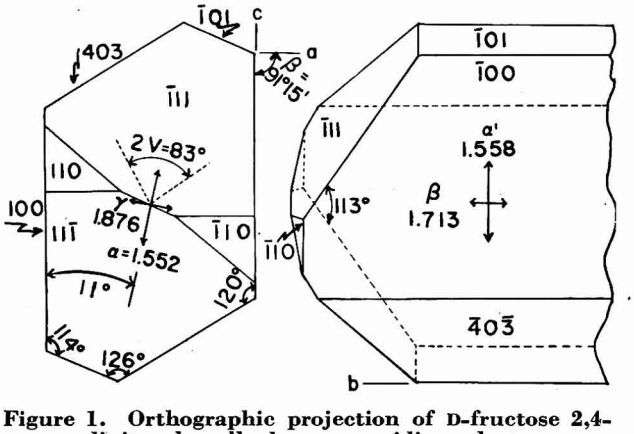

Figure 1. Orthographic projection of D-fructose 2,4-dinitrophenylhydrazone pyridine solvate

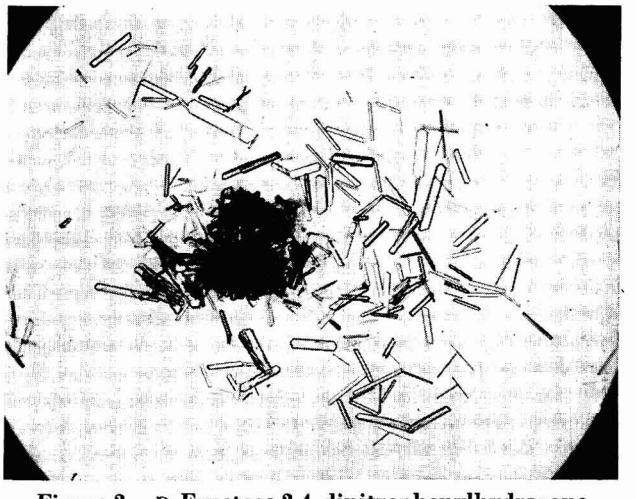

Figure 2. D-Fructose 2,4-dinitrophenylhydrazone pyridine solvate.

100×

Formula Weights per Cell. 2 (2.011 calculated).
Formula Weight. 439.38.
Density. 1.4413 grams per cc. (density gradient tube).

X-Ray Powder Diffraction Data

The camera radius was 7.181 cm. λ for CuKα = 1.5418 A. and a nickel filter was used. The relative intensities were visually determined with a calibrated intensity scale.

d, A.	I/I_1	d, A.	I/I_1	d, A	I/I_1
10.08	0.13	3.49	0.14	2.201	0.04
8.78	0.57	3.42	0.29	2.159	0.04
8.09	0.50	3.28	1.00	2.042	0.05
6.69	0.36	3.12	0.06	1.999	0.03
5.53	0.71	3.07	0.36	1.942	0.04
5.47	0.32	2.884	0.09	1.907	0.04
5.17	0.32	2.778	0.04	1.876	0.04
4.82	0 53	2.698	0.06	1.835	0.03
4.68	0.86	2.646	0.04	1.800	0.02
4.39	0.29	2.584	0.04	1.752	0.02
4.31	0.71	2.518	0.07	1.679	0.02
4.01	0.86	2.438	0.07	1.643	0.02
3.92	0.09	2.393	0.06	1.591	0.02
3.78	0.09	2.333	0.06	1.500	0.02
3.64	0.13	2.248	0.08	1.436	0.02

Optical Properties

Refractive Indices (5893 A.; 28° C.). $\alpha = 1.522 \pm 0.001$. $\beta = 1.713 \pm 0.002$. $\gamma = 1.876 \pm 0.004$. $\alpha' = 1.558 \pm 0.001$ on (100).

Optic Axial Angles (5893 A.; 28° C.). $2V = 83°$ observed; $2V = 82°$ calculated from α, β, ν.

Dispersion. ($r > v$) $2V = 85°$ red (Wratten F), 80.5° green (Wratten B).

Optical Character. (−). From Figure 1 it can be seen that a crystal lying on {100} will show an obtuse bisectrix interference figure and that a crystal lying on {403} will show an optic axis figure.

Optic Axial Plane. (010).

Acute Bisectrix. α.

Extinction. $\alpha \wedge c = 11° \pm 1°$ in acute β.

Pleochroism. Slightly darker yellow for lengthwise than for crosswise vibrations on the ordinary view lying on {100}.

Fusion. Melting point 173–175° C. with decomposition (*1*), odor of pyridine, black bubbly liquid.

ACKNOWLEDGMENT

The authors wish to acknowledge the help given by K. J. Palmer with the x-ray work and by Geraldine Secor with the preparation and analysis.

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MEETING REPORTS

Society for Applied Spectroscopy

THE Society for Applied Spectroscopy met May 12 and 13 in New York, when the following papers were presented:

New Instrument Combination for Optical Emission Spectrochemistry, the Quantograph and Multisource. B. R. Boyd and M. F. Hasler, Applied Research Laboratories, Glendale, Calif.

Three modes of recording optical spectra have been developed over the years—the photographic, the parallel direct-reading method, in which there are a phototube and integrator for each spectrum line, so that a large group can be measured simultaneously, and the sequential direct-reading method, in which a single phototube and integrator are used to measure a series of spectrum lines one after the other. Each mode has special advantages for certain types of spectrochemical analysis.

The quantograph combines all three modes in a single instrument which allows each to be instantly available without any external change in the unit. Thus the instrument is spectrograph, spectrometer, and monochromator, all in one.

In turn this combination optical instrument has been brought together with a new combination source unit of table height to provide the most versatile combination of spectrochemical instruments devised to date. This source unit is a new "high-voltage engineered" version of the Multisource.

To complete the direct-reading instrument combination, the Quantograph and Multisource are coupled with the new recording console of the standard Quantometer.

Typical examples were given of the wide range of spectrochemical problems which can be handled by this combination instrument.

New Equipment and Methods for Emission Spectrographic Analysis. Richard K. Brehm, Jarrel-Ash Co., Newtonville, Mass.

A discussion of several new instruments recently introduced by the Jarrell-Ash Co. was presented. Applications of this equipment to certain difficult analytical problems were discussed.

Diesel Lubricating Oil Analysis for Wear Metal Pick-Up on the Baird Associates Spectromet. L. O. Eikrem and J. S. Fipphen, Engineering Department, Baird Associates, Inc., Cambridge, Mass.

The Spectromet is a new direct reader introduced this spring. It is a lower cost instrument designed for use under conditions normally considered unsatisfactory for the operation of a direct reader. No air-conditioning or humidity control of the locale is necessary. The performance of the instrument in the analysis of Diesel lubricating oil has been tested.

The paper first discussed briefly unusual construction features of the Spectromet. These include the "sealed-in tube" construction and the servo-monitor system which provides continuous and automatic alignment of the spectrum lines with the exit slits. Performance of the instrument in the analysis of Diesel lubricating oils both by the rotating disk and by the ashing techniques was demonstrated. Results on the same samples by the two methods were compared. Both methods use a sample of total light as the reference in place of the more usual added internal standard. With the rotating disk, acceptable analytical results for the control of a Diesel preventive maintenance program have been achieved to concentrations as low as 1 p.p.m. for iron, chromium, aluminum, lead, copper, and silicon and 0.1 p.p.m. for silver. Precision data at these concentration levels were shown.

Design and Performance of the Dual Grating Spectrograph. Robert J. Meltzer, Scientific Bureau, Bausch & Lomb Optical Co., Rochester, N. Y.

The dual grating spectrograph uses two plane gratings which may be rotated independently to photograph two different spectral ranges on one plate at the same time. Two different gratings are employed, one giving 4 and the other 8 A. per mm. in the first order.

The spectra from each of the gratings may be separated on the plate by any desired amount up to 50 mm. to allow two different plates to be used, each in its most sensitive region of the spectrum. The mechanism that keeps the separation constant independent of grating rotation was described.

The grating blaze conditions to give maximum utility of the dual grating system were discussed, and efficiency curves of the gratings were shown. The special problems of illuminating a dual grating spectrograph and the equipment that solves those problems were discussed.

Spectroscopic Excitation. J. T. Rozsa and L. E. Zeeb, National Spectrographic Laboratories, Inc., Cleveland, Ohio.

Development of high-speed cathode ray oscilloscopes provides a greater opportunity for observing excitation phenomena at the analytical gap and thus affords a better understanding of base excitation processes.

Infrared Microspectroscopy. C. R. Bohn, E. I. du Pont de Nemours & Co., Wilmington, Del.

Two topics discussed based on a year's experience with the commercial Model 85 Perkin-Elmer microscope attachment. The treatment is particularly directed to the study of long, narrow samples such as yarn filaments.

The first topic was concerned with "spectral dilution" (energy reaching the detector, but which has not previously passed through the sample). The second was a presentation of the principle of "optimum dispersion" as opposed to maximum dispersion.

Some Chemical Applications of Nuclear Magnetic Resonance Spectroscopy. W. D. PHILLIPS, E. I. du Pont de Nemours & Co., Wilmington, Del.

The techniques of nuclear magnetic resonance spectroscopy permit characterization of the electrical and magnetic environments in molecules of nuclei that possess magnetic moments such as hydrogen, fluorine, phosphorus, and nitrogen. These data can be interpreted in many instances in terms of structures, rate processes, and equilibria of molecular systems. For example, the proton magnetic resonance spectra of *N,N*-dimethylamides prove the existence of restricted rotation involving high energy barriers about the C-N bonds of amides. From analysis of the proton magnetic resonance results, an upper limit of 38 sec.$^{-1}$ can be assigned to the rotational frequency about the C-N bond up to temperatures of 100° C. Another chemical application of nuclear magnetic resonance has been the study of keto-enol tautomerism such as exists in acetylacetone. Jarrett, Sadler, and Shoolery, *J. Chem. Phys.*, 21 2092 (1953).

X-Ray Methods in Chemical Analysis. H. A. LIEBHAFSKY AND P. D. ZEMANY, Research Laboratory, General Electric Co., Schenectady, N. Y.

In the past, x-rays have been important because their diffraction by crystalline materials has made possible the determination of structure. Currently, they are finding increasing application because of three analytical methods based upon x-ray absorption—absorptiometry with polychromatic beams, absorptiometry with monochromatic beam, and emission spectrography, which is currently turning out to be a powerful method for the determination of traces. These three analytical methods were described, and representative applications of each were cited.

Present and Future Industrial Applications of Ultraviolet Spectrophotometry. ROBERT C. HIRT, Stamford Laboratories, Research Division, American Cyanamid Co.

During the "photoelectric era" since 1940, ultraviolet absorption spectrophotometry has demonstrated its usefulness in applications to problems of identification, of physical chemistry, of molecular structure, and of quantitative analysis, even of multicomponent systems. The combination of the high absorptivities of conjugated molecules and a variety of highly transparent solvents has permitted quantitative analyses down to concentrations of parts per million. The ability to observe the spectra of ions in solution has been of great usefulness, both in molecular structure studies and the determination of ionization constants. Applications have been made to such varied problems as solubility, vapor pressure, and industrial hygiene.

The era since 1950 may be considered the "photomultiplier and recorder" era. The introduction of scanning instruments has permitted all the earlier functions to be performed more quickly, and has led to the devising of semiautomatic procedures. Several excellent recording spectrophotometers are now marketed. Most recently, the availability of high quality fused silica for windows and prisms has permitted the extension of the ultraviolet range from 2100 A. down to 1850 A. This extension will be very valuable, for it brings into the useful range certain two-conjugated double-bond molecules and certain compounds having isolated double bonds, as well as making available the much more intense "second" transition bands of aromatic molecules.

The promising aspect of the future lies in the exploitation of the vacuum ultraviolet region, where many more molecules show intense absorption.

With the attachment of lead sulfide detectors to ultraviolet-visible spectrophotometers, the little-used region between the visible and the "rock-salt" infrared, called the "near infrared," is opened to use. Development of this region will probably be done jointly by the infrared and the ultraviolet-visible spectroscopists, because of the overlap of their interests and instrumentation.

Recent Developments Extending the Industrial Applications of X-Ray Spectroscopy. B. R. BOYD AND J. W. KEMP, Applied Research Laboratories, Glendale, Calif.

New instruments have been developed which increase the ultimate practical precision which can be obtained by x-ray emission analysis. These use reflecting curved crystal spectrometers to give high intensities.

The design of several types has now been completed, each for different needs. A semifixed focus type which can be nested and, hence, used in groups, has been developed for the x-ray industrial Quantometer. This instrument is admirably suited for high-speed, repetitive x-ray determinations as might be required for industrial production control. A scanning type, too, has been developed. It is particularly suited for analytical research work.

The performance of these spectrometers has been studied in the analyses of a variety of materials—alloys, ores, slags, oils, and catalysts. Quantitative methods have been developed which allow for correction of sample conditions and interelement effects.

Boron Trichloride Determination by Infrared Absorption Spectrophotometry. HARRY M. BOWMAN AND DAVID M. GARDNER, Chemistry Department, Reaction Motors, Inc.

The infrared absorption spectrum of boron trichloride has been obtained in the sodium chloride region at two path lengths and at several pressures. The ordinate function of the spectrum can be used for measuring the boron trichloride content in a vapor phase product at most of the absorption maxima in the spectrum over discrete sample pressure ranges.

A singular exception to this generalization, in the 2- to 15-micron wave-length region, appears at 7.26 microns, an absorption maximum which is ascribed to the in-phase B-Cl bending vibration. Absorbance varies nonlinearly with pressure.

Spectrophotometric Study of the Hydrolysis of Chlorine. FREDERICK C. STRONG III, Stevens Institute of Technology, and GEORGE L. ZIMMERMAN, Bryn Mawr College, Byrn Mawr, Pa.

This spectrophotometric investigation of aqueous chlorine is an interesting example of the application of a spectrophotometer to a system that has been studied principally by other means. The equilibrium constant for the reaction:

$$Cl_2 + 2H_2O = H_3O+ + Cl- + HClO$$

is important in the calculation of oxidation potentials. The current literature value was obtained by recalculations of measurements of electrical conductivity made over 50 years ago. By means of a spectrophotometer and special techniques, the authors were able to obtain a better value for this constant. Verify that vacuum distillation is a satisfactory method for preparing pure hypochlorous acid, accurately determine the absorption spectra of hypochlorite ion, hypochlorous acid, and aqueous chlorine in the ultraviolet and visible regions and determine the effect of solvation on the absorption spectrum of chlorine.

Use of a Direct Reading Spectrometer for Inspection and Quality Control. W. L. WRIGHT, Hamilton Standard Division, United Aircraft Corp., and J. H. Jurmain-Baird Associates, Inc.

Methods used to overcome some of the obstacles presented by sample shape and size were discussed, together with steps taken to increase flexibility of the instrument. A method of analysis for foil samples 0.003 inch thick was described.

Factors Affecting Accuracy in the Spectrochemical Analysis of High Temperature Nickel Alloys. R. W. LOOFBOUROW AND M. A. HARRINGTON, Utica Drop Forge and Tool Corp., Utica, N. Y.

The composition and physical properties of the nickel-base "super" alloys impose a variety of factors which complicate the spectrochemical analysis of these materials. It has been found that such factors as the surface, mass, temperature, and metallurgical history of the samples must be explored with great care in order to ensure reasonable accuracy in the analytical results. In the work done here a standard deviation of 0.05 has been maintained in the determination of titanium in the range of 3.00% and approximately the same value for aluminum in the range of 1.25%.

Semiquantitative Spectrochemical Analysis Using Spex Standards. A. J. MITTELDORF AND D. O LANDON, Consolidated Testing Laboratory, New Hyde Park, N. Y.

Spex standards consist of a matrix (such as graphite, zinc oxide, or aluminum oxide) in which are accurately dispersed a large number of common elements, each at the identical concentrational level. Recommendations for using the standards, the degree of accuracy to be expected, the scope, and limitations of the method and illustrations of typical analyses were described. Among the materials whose analyses were illustrated were a number of NBS standards, fluxes, and organic ashes.

Direct-Reading Intensity Photographic Photometer. MILTON GREEN, Signal Corps Engineering Laboratories, Fort Monmouth, N. J.

An existing densitometer has been modified so that it can record or indicate transmittance, density, or logarithm of exposure intensity corresponding to a given photographic density. A 1P21 photomultiplier is employed as the detector. Conversion from transmittance to density is accomplished with the aid of nonlinear circuit elements. The conversion of density to logarithm of intensity is obtained by employing the cathode current *vs.* grid voltage characteristic of a 5692 triode with appropriate parameters as an electrical analog of the H & D curve.

Society for Analytical Chemistry

AT A MEETING of the Midlands Section held April 6 in Birmingham, B. Bagshawe, Brown-Firth Research Laboratories, Sheffield, spoke on "The Analytical Chemistry of Niobium and Tantalum, with Particular Reference to Steel and Allied Materials."

The chemical properties of niobium and tantalum were enumerated and their reactions of analytical importance were described, including precipitation from ferrous metallurgical solutions and determination in steels and ferroalloys. The separation of niobium and tantalum from titanium, tungsten, and each other was discussed. Descriptions were also given of nascent hydrogen reduction, and chromatography and color reactions—thiocyanate, pyrogallol—of niobium and tantalum.

At the meeting of the Scottish Section, of the Society for Analytical Chemistry held April 28 in Edinburgh, three papers were presented and discussed.

Determination of Small Amounts of Zinc in Various Materials. J. A. HUNTER, University of Edinburgh, Edinburgh, Scotland.

The disodium salt of ethylenediaminetetraacetic acid has been applied to the determination of small amounts of zinc that have previously been separated from many other elements by means of an anion exchange procedure. Solochrome black was used as indicator, but irregularities were encountered when the end point was located visually, and the titration was therefore studied spectrophotometrically. As a result, amounts of zinc ranging from 100 γ to 5 mg., contained in about 100 ml. of solution, have been determined with an accuracy of ± 10 γ.

When lead is present, a special titration procedure is required. Interference with the titration, which is encountered from small amounts of tin, indium, gallium, and uranium that have escaped separation via the anion exchange resin, may be eliminated by solvent extraction of zinc pyridine thiocyanate before titration. This procedure also largely eliminates lead.

The method has been applied, with good precision, to the determination of zinc in 100-mg. samples of a few alloys and a borosilicate glass, and would appear to be applicable to a wide range of materials containing zinc.

Determination of Calcium and Magnesium in Plant Material Using Disodium Ethylenediamine Tetraacetate (EDTA). E. S. R. MCCALLUM AND A. M. SMITH, Edinburgh and East of Scotland College of Agriculture.

The method involves two titrations, one for calcium and magnesium using Solochrome black as indicator, and the other for calcium alone using murexide as indicator.

The iron manganese, and phosphate in extracts of plant ash interfere in one or both of the titrations and must be removed. Phosphate is removed by double precipitation at pH 3 to 4 by adding an excess of 10% aqueous ferric chloride to the hydrochloric acid extract and adjusting the pH with ammonium hydroxide and acetic acid until the solution is just acid to methyl red. The filtrate is evaporated to 150 ml. and made just alkaline to methyl red, and the manganese is precipitated as MnO_2 using either ammonium persulfate or bromine water as oxidizing agent. The resulting solution is made up to a known volume and aliquots are used for titration with EDTA.

Excess of ammonium chloride affects the titration for calcium, no end point being observed with murexide, and must therefore be removed by boiling with sodium hydroxide prior to titration. For quantities of the order of 1 to 7 mg of calcium and magnesium oxides, the method gives results for synthetic solutions to within 2%, the calcium tending in general to be slightly low and the magnesium slightly high. For plant materials, results compare favorably with those obtained by the conventional methods, and the recovery of magnesium is good.

The method is faster than the conventional methods, but the number of samples that can be conveniently handled simultaneously is smaller.

Chromatographic Separation and Determination of Alkaline Earth and Alkali Metals. J. B. HEADRIDGE AND R. J. MAGEE, University of Edinburgh.

Chromatographic investigations into the separation of the Group I and II elements of the periodic table have been made using a wide variety of solvent mixtures. Lithium, sodium, and potassium are easily separated, but the separation of potassium, rubidium, and cesium, and of beryllium, magnesium, calcium, strontium, and barium presents *greater* difficulties. Particular attention has been given to variation in the quantities, as a solvent mixture, which separates equal amounts of the cations, frequently *fails to* produce satisfactory results when the quantities are widely varied.

Butyrates, chlorides, and nitrates of the elements have been used. A solvent mixture has been evolved which will separate 10 micromoles of the Group II cations into the individual constituents, in cases where the minimum amount under consideration is 0.1 micromole. This solvent mixture has been successfully applied to the semiquantitative analysis of carbonate rocks and grass samples. Attempts to obtain more quantitative determinations of magnesium and the alkaline earths, separated by paper chromatography, have been made by cutting out the separated salts, treating the paper, and finding the amount of each cation by titration.

The results of investigations carried out to obtain a solvent mixture that will separate all the alkali-metal cations on one strip were also presented.

At a meeting of the Biological Methods Group held May 13 in London, several papers were presented and discussed.

Microbiological Estimation of Vitamin B_{12} in the Serum. R. H. GIRDWOOD, Department of Pathology, University of Edinburgh.

The serum level of vitamin B_{12} may be measured with *Euglena gracilis* or *Lactobacillus leichmannii* as the test organism. An account was given of experiences with the *L. leichmannii* method, which is not suitable for a routine laboratory. Important factors are the concentration of acetate buffer in the medium and the addition to it of thioglycolic acid or other reducing agent. With a satisfactory technique, false positive and false negative results have not been encountered.

Clinically it is possible to diagnose pernicious anemia by estimation of the serum vitamin B_{12} level, and when megaloblastic anemia is due to malabsorption of vitamin B_{12} as well as folic acid (as in a small proportion of cases of idiopathic steatorrhea) this can be shown by estimation of the serum vitamin B_{12} level.

Estimation of Vitamin B_{12} in Animal Feeding Stuffs Using *Lactobacillus leichmannii* and *Ochromonas malhamensis* as Test Organisms. D. H. SHRIMPTON, Rowett Research Institute, Bucksburn, Aberdeen.

The successful use of these methods in assaying the vitamin B_{12} activity of feeding stuffs depends upon the development of large scale assays without loss of precision. A rapid method of setting up assays by using semiautomatic dispensing equipment was described, and also a shaking machine, which is used with vitamin B_{12} activity determined with *Ochromonas malhamensis*.

In many feeding stuffs a higher vitamin B_{12} activity has been found when *L. leichmannii* was the test organism than when *O. malhamensis* was used. These values were tested and their interpretation was discussed.

Estimation of Vitamin B_{12} in Milk. MARGARET E. GREGORY, National Institute for Research in Dairying, Shinfield, Reading, Berks.

Vitamin B_{12} occurs in the milk of the human, cow, goat, sow, ewe, and rat in a bound form, which presents some special assay problems. For example, vitamin B_{12} can be measured quantitatively in cow's milk after dilution of the milk with water and heating with the assay mediums, but for human and sow's milks this simple treatment is not adequate. With milks of different species, different extraction methods and assay techniques (those using *L. leichmannii* ATCC 4797, *Bacterium coli*, and *O. malhamensis*) have been compared and these results were presented and discussed.

Biological Methods of Estimating Vitamin B_{12}. MARIE E. COATES, National Institute for Research in Dairying, Shinfield, Reading, Berks.

Biological methods of assay of vitamin B_{12} are usually based on the growth response of chicks or rats given all-vegetable diets. Thus they are a measure of total "animal protein factor" activity rather than of vitamin B_{12} alone. They may fail to detect vitamin B_{12} in some natural materials when it occurs in bound forms. Assay results are unlikely to be complicated by the presence of vitamin B_{12}-like factors, as few of them have appreciable growth activity for animals.

Critical Analysis of the Method of Vitamin B_{12} Assay Using *Euglena gracilis* as Test Organism. W. R. PITNEY, Post Graduate Medical School, Hammersmith, London.

Observations have been made on the variation in growth response of *Euglena gracilis* to standard vitamin dilutions, the reproducibility of results from repeated assays of sera, and the recovery of vitamin B_{12} added to serum in vitro. The significance of "free" and "bound" vitamin B_{12} in serum and tissues has been investigated by dialysis and electrophoretic methods.

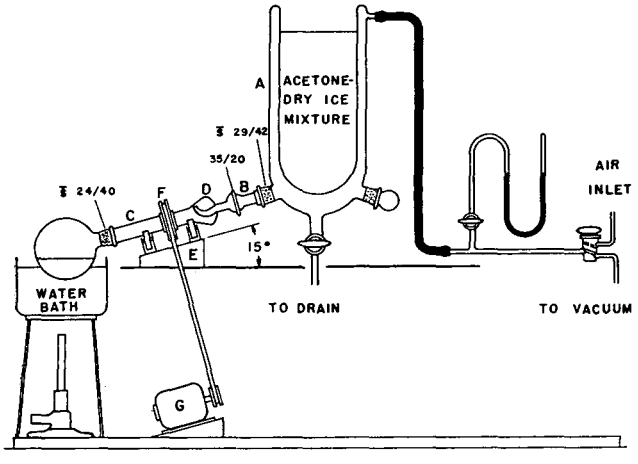
An All-Glass Rotary Film Evaporator

Murray E. Volk, Nuclear Instrument and Chemical Corp., Chicago 10, Ill.

SEVERAL devices have been described which utilize the principle of evaporation from a rotating film under vacuum for concentrating solutions of heat-sensitive materials. A simple all-glass apparatus which employs this principle has been developed. In this device connection is made from a rotating round-bottomed flask to a commercially available lyophilizing unit through a lubricated ball joint. Since the axis of rotation lies approximately 15 degrees from the horizontal, somewhat more than one half of the volume of the round-bottomed flask may be occupied by the solution when the evaporation is started without danger of mechanical transfer. The turbulence induced in the liquid by the rotatory motion of the flask effectively prevents bumping due to local superheating. Aqueous and ethanolic solutions in flasks of 2 liters in capacity have been evaporated to dryness in 1 hour with this device.

The figure illustrates the apparatus. The lyophilizing unit, *A*, 18 × 50 cm. in outside dimensions is firmly mounted on a table. Into one of its six outer 29/42 standard taper joints is fitted a short adapter, *B*, carrying the ball portion of a 35/20 ball joint. The hollow axle, *C*, is fabricated of 22-mm. outside dimension glass tubing and carries the socket part of the ball joint at its elevated end, and an inner 24/40 standard taper joint at its lower end, to which the round-bottomed evaporation flask may be attached. The Kjeldahl trap, *D*, while included in the design, has not proved essential to satisfactory operation. The entire hollow shaft is 29 cm. long. The bearing, *E*, consists of four rubber wheels mounted in pairs and serves to support the weight of the rotating assembly and to absorb any vibration. The drive is a metal pulley, *F*, with a center hole bored somewhat larger than the glass shaft. The pulley is fastened to the shaft by four Allen screws which make contact with a split-brass cylinder. The two halves of the brass cylinder are cushioned from the glass tubing by a layer of sponge rubber. A V-belt of suitable dimensions drives the pulley. A $^1/_{16}$-horsepower motor, *G*, equipped with a speed reducer turning at approximately 40 r.p.m., rotates the assembly. A variable source of power may be employed to control the speed of rotation, but its use has not been necessary. Only Apiezon N has proved suitable as lubricant for the rotating ball joint. A vacuum pump capable of maintaining pressure below 0.5 cm. of mercury is adequate for operating the apparatus. During operation, the pressure differential is sufficient to hold the evaporation flask to the rotating axle, although spring clips may be used.

This design for a device for the concentration of heat-sensitive materials using evaporation from a rotating film under vacuum possesses several points of superiority over others previously described. The apparatus of Craig, Gregory, and Hausmann [ANAL. CHEM., 22, 1462 (1950)] requires the rotation of both the evaporation flask and the condenser bulb. The inherent high inertia of this system makes accidental breakage of the apparatus during operation very possible. Partridge's device [Partridge, S. M., *J. Sci. Instr.*, 28, 28 (1951)] rotates a long-necked flask and employs only a single bearing, but the vacuum take-off is complicated, requiring special machining and packing of the vacuum gland. Only the single hollow shaft and the evaporating flask rotate in the device presented here. Use of a lubricated ball joint as the rotating vacuum connector makes construction simple and straightforward. The entire apparatus can easily be fabricated without outside assistance using available standard components.



A condenser of spherical shape such as employed in the apparatus of Craig and coworkers performs its function in a relatively inefficient manner. The ratio of condensing surface area to total volume of the condenser is minimal. The work of this laboratory involves the concentration of solutions containing considerable quantities of radioactivity. The lyophilizer used in the authors' apparatus has been found to act as an efficient condenser which ensures that any radioactivity in solution, which might be carried mechanically into the condenser, is trapped and does not escape into the pumping system and possibly contaminate the laboratory.

While a commercial lyophilizing unit such as this device employs is a relatively expensive piece of equipment, it is in use in many laboratories working with biological extracts and other heat-sensitive materials. The alternative function suggested here, as a condenser for a liquid concentrating device, which use in no way interferes with its original purpose, serves to increase its usefulness.

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Matched Test Tubes in the Beckman DU Spectrophotometer

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LARGE sets of matched test tubes are a great convenience for colorimetric analysis. Tedious rinsing of photometer cells is no longer required when a dry tube is available for each sample or standard color. The light path of 2-cm. length which may be obtained with 10 ml. of solution is more convenient in many cases than that of the usual 1-cm. cells. Little time or expense is required to prepare such tubes, and the accuracy of analysis is not noticeably affected by the slight variation of 2 or 3 parts per thousand in the diameters of the tubes of any one set. Only a single day's time was required to produce four matched sets comprising 200 tubes. The installation of the tube holder does not require the removal of the regular cell holder and does not impair the use of the instrument with the regular cells. In this latter case, a blackened cork is used to seal the test tube opening. This report describes the adaptation of the Beckman DU spectrophotometer for this purpose, and a simplified method of matching the tubes.

The tube holder was prepared from a hardwood board by boring holes as shown in Figure 1. Care was taken to maintain true alignment and accuracy, and the two large surfaces were made plane and parallel. The size of the hole may be adjusted to fit the tubes at hand snugly and yet freely. The lower part of the tube was centered by its round bottom resting on the ledge at the bottom of the hole. The lower $^5/_8$-inch hole provided

drainage in case of accidental spillage, and was closed with a blackened cork inserted from underneath. A 6 × 1.5 inch cardboard mailing tube was used as a tube cover. The holder and cover were finished with a dull black paint.

The holder may be inserted between the cell holder provided with the instrument and the photocell compartment, or in place of the original cell holder if the latter is no longer required. In either case, a new set of longer screws is required to reassemble the apparatus, the proper length being 5.75 or 5 inches, respectively. These four screws may be improvised by threading a short distance at both ends of rods with an 8-32-NC die, and screwing a knurled battery post nut tightly on one end. After assembly, the holder and cover may be tested for light leaks by turning the instrument switch on and setting the density scale to infinity, keeping the lamp off. When the photocell shutter switch is opened and closed, there should be no discernible motion of the meter pointer.

Two gross of 22 × 175 mm. test tubes with lip, Kimble Catalog 45050, were procured for matching. The tubes were examined carefully, and a small number rejected for being off-color or too large to enter the hole in the holder freely. The remainder were cleaned with dichromate–sulfuric acid and dried. A convenient solution for matching the tubes at a wave length of 373 mμ was made by diluting 8 ml. of 10*N* sodium hydroxide and 6.38 ml. of 0.1*N* potassium dichromate to 2 liters. This solution was adjusted so that it gave an absorbance (optical density) of exactly 0.500 when compared with distilled water in a 1-cm. cell. The strength of the solution as prepared was 2.3% greater than the theoretical value [Haupt, G. W., *J. Research Natl. Bur. Standards*, 48, 414 (1952)] to allow for this adjustment and for possible losses due to reduction of the chromate by impurities. Ten milliliters of the colored solution were then placed in each test tube, and the absorbances were measured as described below. These figures when multiplied by 2 gave the effective diameters of the tubes in centimeters.

The measurements revealed the fact that less than 10% of the tubes were perfectly round. Hence, each tube was rotated and the major and minor axes and density readings were marked with a crayon. To facilitate the handling of the large numbers of tubes, an open-top shield was used in a darkened room, with a shielded light for the meter scale. This left the protruding tops of the tubes free for turning and marking.

In order to use the most accurate portion of the instrument scale (the low density end), all densities were measured relatively against one selected round tube as a reference, which was always used in one marked position. This selected tube, containing 10 ml. of the adjusted chromate solution, was placed in the holder, and the instrument was balanced against the scale set at zero absorbance or 100% transmittance (switch in 1 position). Various settings of the sensitivity control were used and the balance was made by adjusting the slit width to bring the meter needle to zero, until the sensitivity setting was found where deflecting the absorbance scale 0.005 from the balanced position resulted in a meter needle deflection of 5 units. The relative densities of large numbers of tubes could then be rapidly and accurately measured within 0.001 by using any fixed convenient density scale setting and correcting this value by the meter needle deflections.

Table I. Matched Groups Obtained from 288 Test Tubes

(Figures are number of tubes)

Group	Minor Axis Diameter, Cm.	Ellipticity, Parts per Thousand 0–3	4	5	6	7	8–9	10–11	Total in Group
A	2.031–2.040	5	5	8	4	5	2	1	30
B	2.020–2.030	10	8	23	9	17	21	9	97
C	2.010–2.019	1	5	9	3	9	11	8	46
D	2.000–2.009	3	4	3	3	5	5	4	27

Table II. Transmittance Characteristics of Kimble Glass Test Tubes

[Absorbance of test tube filled with distilled water *vs.* air (no tube) as reference]

Wave Length, Mμ	Density	Wave Length, Mμ	Density	Wave Length, Mμ	Density
350	0.077	550	0.033	750	0.058
375	0.050	575	0.030	775	0.058
400	0.042	600	0.033	800	0.055
425	0.045	625	0.037	825	0.055
450	0.043	650	0.035	850	0.071
475	0.040	675	0.036	875	0.078
500	0.036	700	0.036	900	0.087
525	0.034	725	0.044		

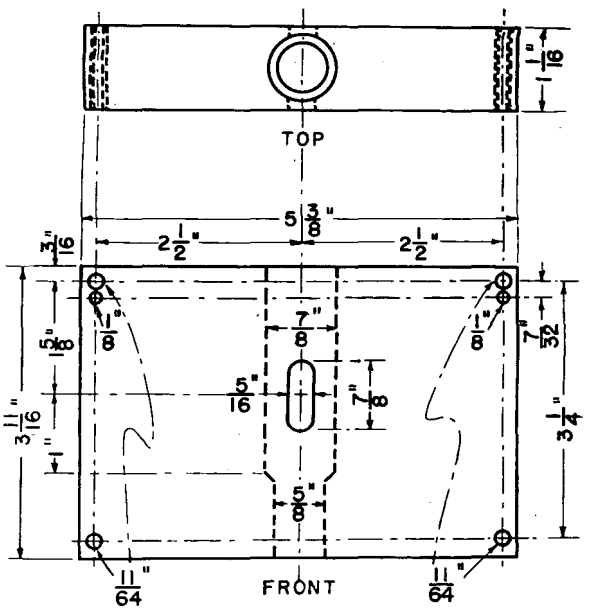

Figure 1. Test tube holder for Beckman DU spectrophotometer

In order to obtain the absolute absorbances, the density of the standard reference tube was then measured in the instrument against a tube of like diameter containing distilled water. This value was later used to compute the effective diameter range of each matched set. The value was added to the readings to give the absolute absorbances of the tubes. The latter figure when multiplied by 2 gave the effective diameters in centimeters.

The marked tubes were then sorted into groups according to their minor diameters (which were considered less affected by flaws in the glass than the major diameters) and their degree of ellipticity. The relative density markings on the tubes gave the differences in parts per thousand directly, as the total absolute absorbances were very close to 1. The groups were chosen so that each set was matched in minor diameters within ±2 to 3 parts per thousand (relative densities matched within ±0.002 to 0.003). The tubes with an ellipticity exceeding 11 parts per thousand (density difference between major and minor axes exceeding 0.011) were rejected.

A tabulation of the results (Table I) shows the four groups finally selected, comprising a total of 200 tubes out of the 288 tested. Each of the selected tubes was permanently marked with the letter of the group to which it belonged, just under the lip in the exact position of the minor axis. When the tubes were used for analytical work, care was taken to position the letter marking so that the light went through the minor axis. Inaccurate positioning was not likely to result in an error of more than 2 to 3 parts per thousand with the most elliptical tubes of each set. It was thus concluded that the sets should match within 0.5%, which is well within the accuracy of usual colorimetric procedures.

In order to know the useful wave-length range in which these tubes could be used, the transmittance characteristics of the glass were also determined. These measurements, given in Table II, are the absorbances of a tube filled with distilled water compared to air (no tube) as a reference. The tubes were found suitable for use over a wide wave-length range.

The tubes have been used for several years, with excellent results. Cleaning with strong chemicals such as dichromate–sulfuric acid or alcoholic potassium hydroxide is kept to a minimum to avoid etching the glass. Generally the tubes are rinsed with distilled water, the outsides wiped dry with a towel, and the tubes dried upright in an oven at 110° C. The tubes have been found to be accurately matched for all the colorimetric procedures used; uniformly straight plots have been obtained.