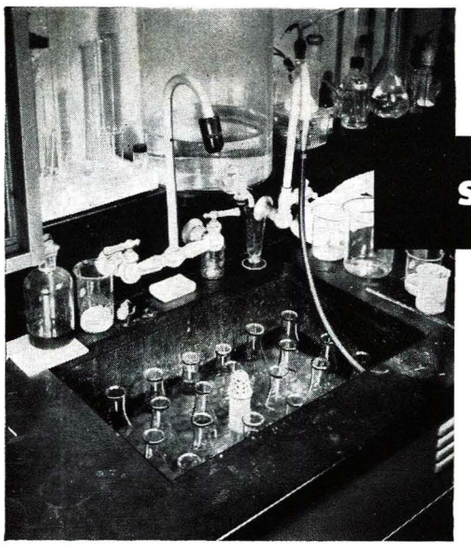
ANALYTICAL CHEMISTRY

FEBRUARY 1951



sink "dammed up"?

SOLVE COOLING PROBLEMS with the FISHER REFRIGERATED BATH

All too often, the laboratory sink is made inoperative with flasks of samples being cooled under running water. This makeshift method is inefficient and a real annoyance which impairs the work of the entire laboratory. The results may also be subject to error because of splashing.

The solution to the problem is, of course, the Fisher Refrigerated Bath — a compact unit with built-in mechanical cooling and thermostatically controlled temperature.

Area of the floor of the bath is 21" by 37½" — sufficient to accommodate sixty 300-ml. Erlenmeyer flasks. Adjustable shelf level, convenient drain, interior of solid copper. Price, $350.00.

Write for literature on the Fisher Refrigerated Bath.

Metallurgical laboratories can save hours of cooling time with the new bath.

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ANALYTICAL CHEMISTRY

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Issued February 16, 1951

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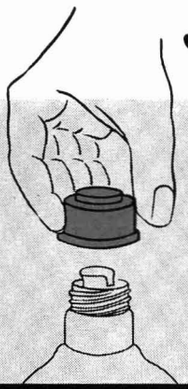
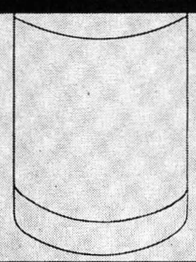
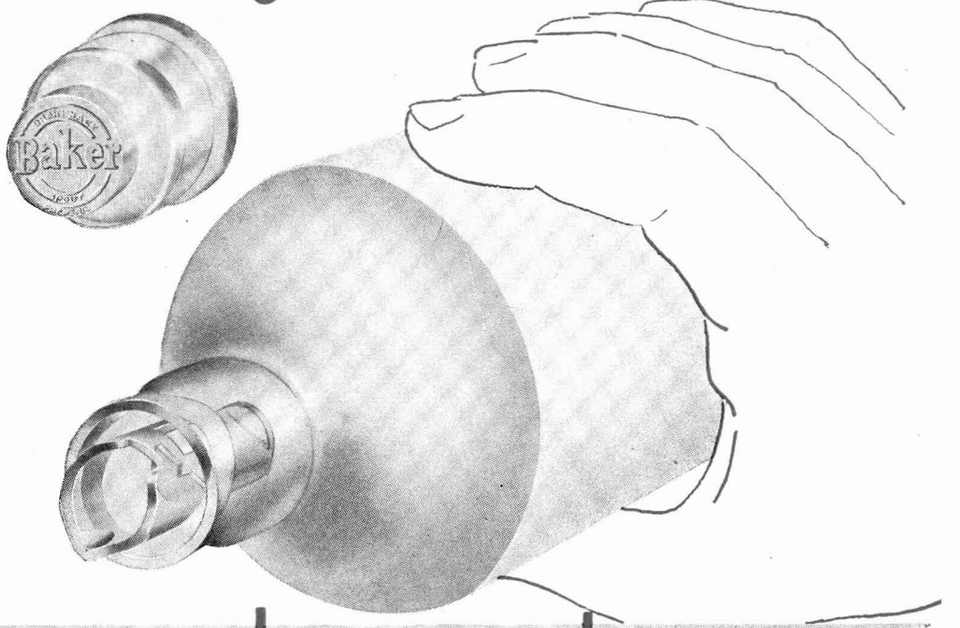
a new POLYETHYLENE package for 'Baker Analyzed' HYDROFLUORIC ACID with these 6 distinctive advantages

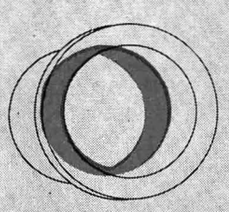


Increased safety with this large size easy-to-remove screw cap.

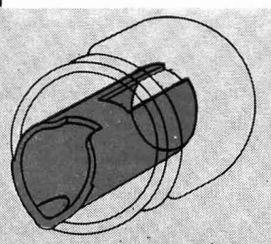


Increased protection against contamination—bottle and pouring tube (both polyethylene) are inert to Baker Reagent 49-52% Hydrofluoric Acid.

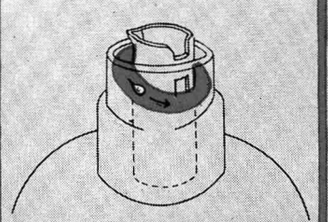




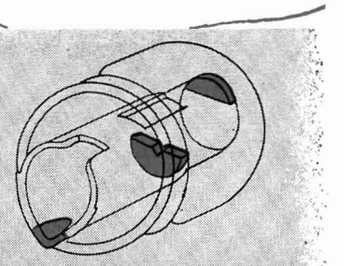
No diaphragm to puncture—no acid spray due to sudden release of pressure in bottle—bottle rim is fitted to groove in cap for tight seal.



Easy pouring when open and safe handling when closed—pouring tube is completely enclosed by cap and outer sleeve of bottle rim.



No acid droplets remaining on lip of pouring tube with this special drain-back channel—added protection for the user.



A drop at a time or even, measured flow. Two baffles—one at the top controls flow of air while pouring—other, at bottom, controls liquid flow.

The development of this polyethylene bottle with its built-in drain-back spout and 6 distinctive features is just another example of the care that Baker takes to safeguard the quality of its Laboratory Chemicals.

Remember, this new bottle makes pouring easy. You can get controlled flow without the danger of acid running down the outside of the bottle.

The next time you order Hydrofluoric Acid specify Baker Analyzed Hydrofluoric Acid in the *new polyethylene package.* If your favorite laboratory supply house does not have the new Hydrofluoric Acid package in stock he will be glad to get it for you.

J. T. Baker Chemical Co., *Executive Offices and Plant:* Phillipsburg, N. J.

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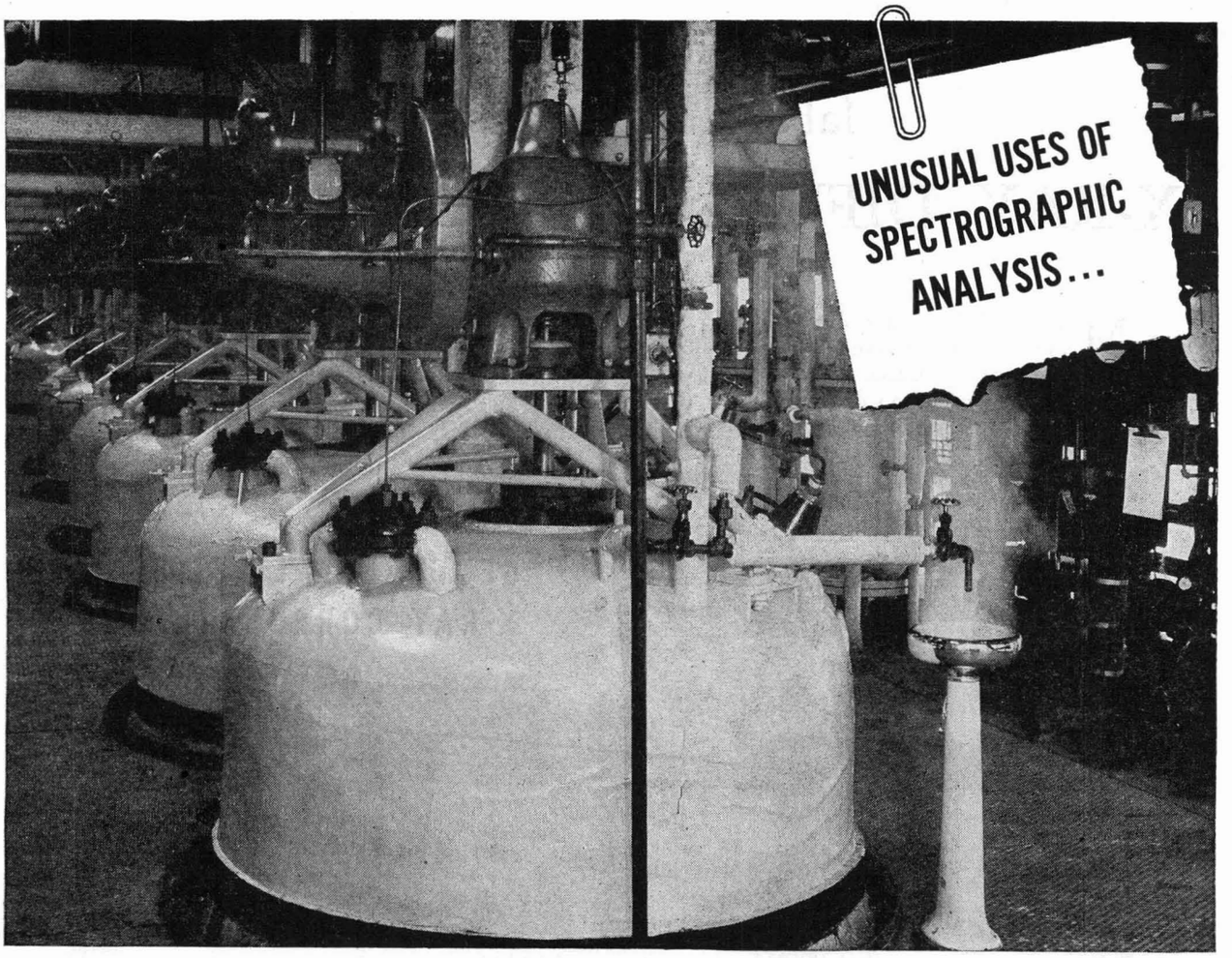

Precision control of trace metals in streptomycin production

Few American organizations enjoy the reputation for undeviating high quality that E. R. Squibb and Sons have built up over the years. Their products often mean the difference between life and death. At Squibb, no compromise is ever made with quality, regardless of trouble or expense.

Squibb uses "National" spectroscopic carbons in their spectrochemical method as one tool in the control of their streptomycin production. The upper limit of trace metals in the finished product of streptomycin must be carefully controlled. Here is real precision quantitative control. If the spectrograph does a job for Squibb, it should be useful in any analysis job requiring precision.

WHY IT PAYS TO USE "NATIONAL" SPECTROSCOPIC ELECTRODES. National Carbon's spectroscopic electrodes are the purest obtainable. Each shipment is accompanied by a "Statement of Purity" which tells your analyst what trace elements are present in the electrodes. As a result, he can assess his plates, or film, very quickly and accurately without being confused by unexpected spectral lines.

OTHER ADVANTAGES OF SPECTROGRAPHIC ANALYSIS

- Sensitive to 1/10,000,000 of a gram for some elements
- Saves time in analysis
- Detects unsuspected metals
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- Provides a permanent record
- Differentiates between two elements chemically very similar
- Analysis can be made in some cases without destroying sample

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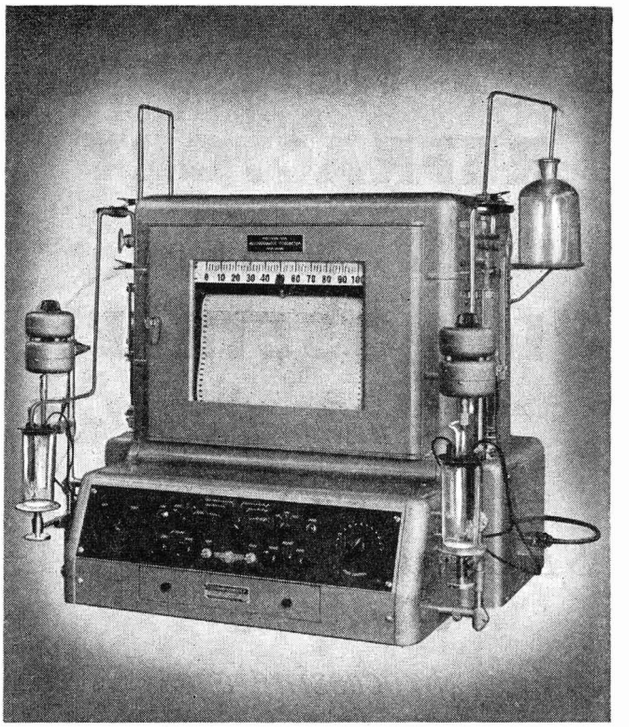

PRECISION-Dow Dual Recordomatic Titrometer is one of the greatest *money-savers* in production control. By saving time of technical personnel and keeping permanent chart record, it has reportedly "paid for itself in 3 months," "cut costs to scarcely ⅓rd," "daily runs 6 times former volume." Bulletin 1-640A

For New ECONOMY in Laboratory Apparatus

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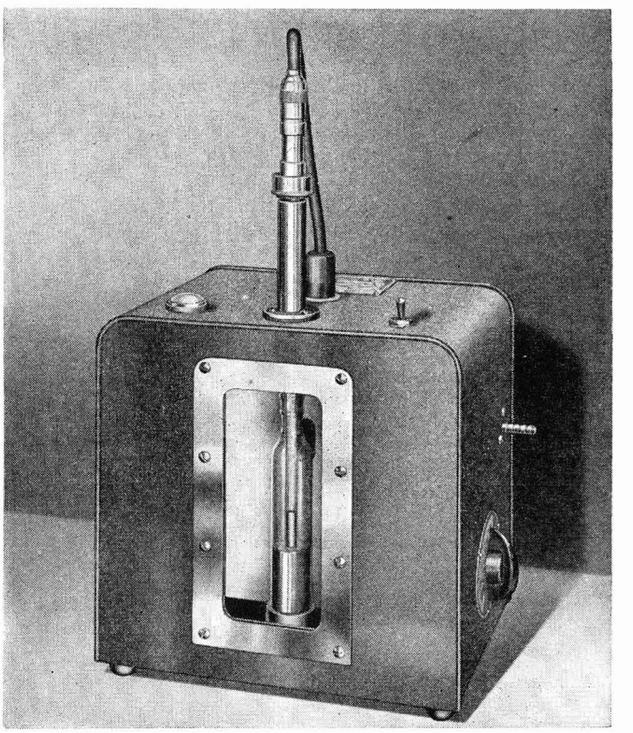
Specify *Precision to be Sure

PRECISION Micro-Set Manostat selects and holds an *exact* (0.05 mm Hg) vacuum from Room to 0.1 mm of Absolute . . . *constantly* without distracting adjustment. Used between pump and distillation system, this instrument is more than *accurate*—finger tip control-setting, compact, notably low priced. Bulletin 1-866



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NEW REFRACTORY DOES THE "IMPOSSIBLE"

Unusual properties of NORTON FUSED STABILIZED ZIRCONIA start new processing era

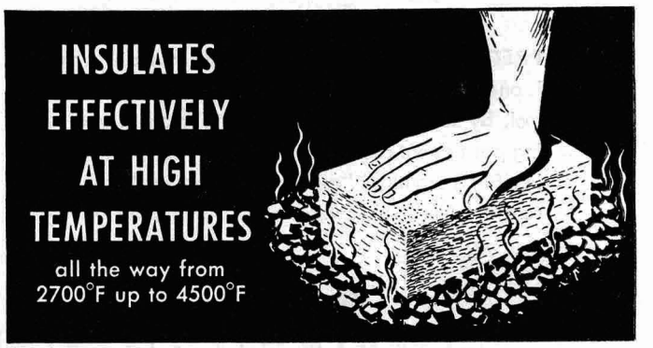
AGAIN . . . Norton Research opens the doors to new technical advances in the processing field. First to produce Fused Stabilized Zirconia in commercially significant quantities, Norton now makes it possible for you to lift your process-temperature ceiling up to 4500°F.

Extraordinary properties

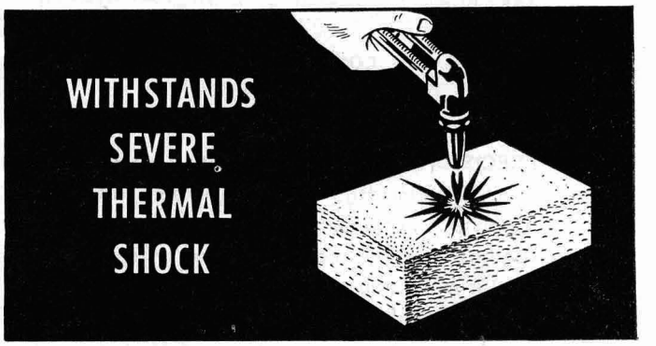
No other refractory offers such an unusual combination of properties. *Resistance to high temperature* comes first, of course. But look at its *low thermal conductivity* . . . surprising because of its relatively heavy weight (twice fire clay's). What's more, Norton Stabilized Zirconia insulating grain has a thermal conductivity of only 3 Btu. Consider, too, its *great strength* at elevated temperatures . . . its ability to *resist oxidizing and reducing atmospheres at high temperatures* . . . its *chemical inertness* in contact with titanates. Even more amazing is the *electrical resistivity* of Norton Fused Stabilized Zirconia . . . ranging from 2300 ohm-cm. at 1300°F down to 0.37 ohm-cm. at 4000°F. Resistance furnace operators take notice.

WRITE FOR DATA. In Technical Bulletin SZ1, Norton researchers and refractory engineers give you all the up-to-the-minute facts about Fused Stabilized Zirconia. Get your copy . . . use the coupon below today.

HIGHLY REFRACTORY, Norton Stabilized Zirconia has successfully speeded up gas synthesis production by permitting operating temperatures as high as 4500°F. Resists *all* oxidizing atmospheres and *most* reducing atmospheres.



LOW THERMAL CONDUCTIVITY of Norton Stabilized Zirconia brick of 6 Btu compares well with 3000°F insulating brick's 6+, fire clay's 12, alumina's 20 and silicon carbide's 50+ (approximate values at 2700°F).



GOOD THERMAL SHOCK RESISTANCE of Norton Stabilized Zirconia to extreme temperature gradients and rapid thermal changes offers interesting new possibilities in all processing industries where high temperature is a factor.

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Gentlemen:
Please send me without obligation, Technical Bulletin SZ1 on Norton Fused Stabilized Zirconia. I'm interested in investigating its use for:

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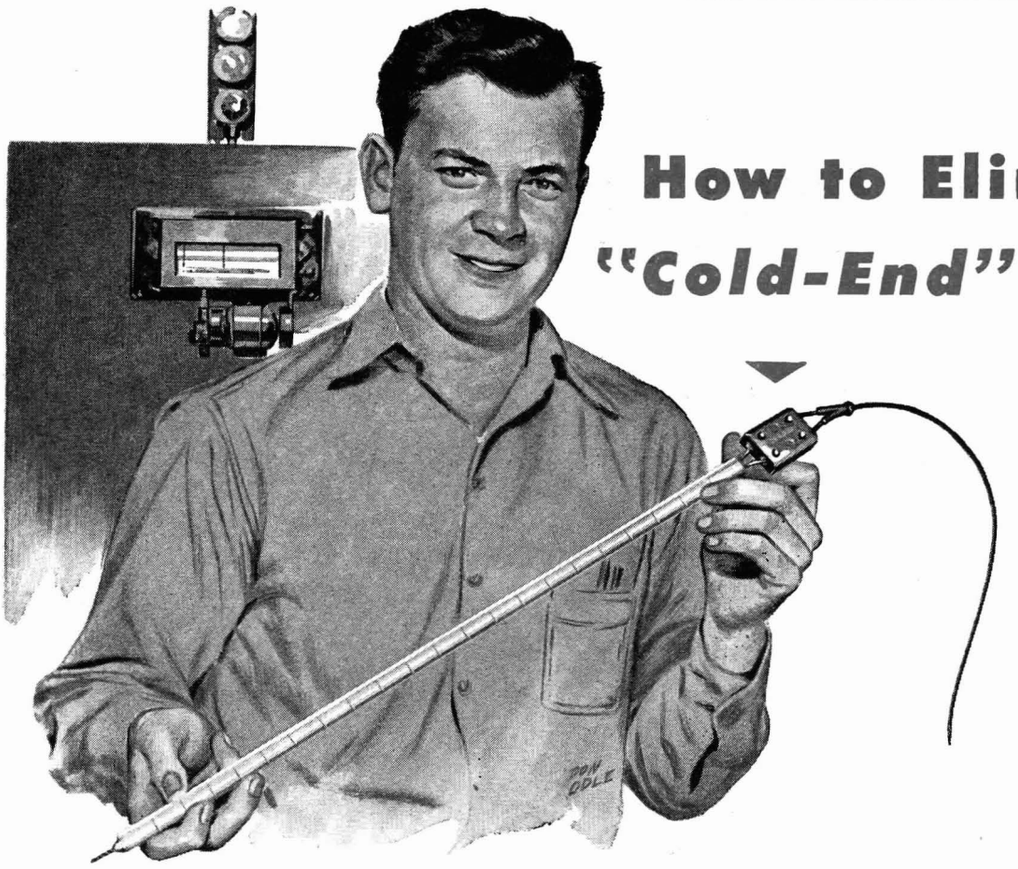


How to Eliminate "Cold-End" Errors

Use Chromel*-Alumel *Extension Leads* with your Chromel-Alumel Thermocouples

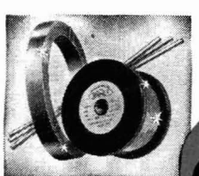
Modern pyrometers, within themselves, leave little to be desired as far as accuracy of temperature measurement is concerned. They're precision built instruments . . . delicately balanced . . . equipped with automatic cold-end compensators . . . designed and engineered throughout to give you the fine degree of accuracy you need. And, on 3 out of every 4 heat treating installations, these accurate meters are calibrated for CHROMEL-ALUMEL thermocouples . . . durable, dependable, sensitive couples unconditionally guaranteed to register true temperature—E.M.F. values within extremely close specified limits.

Yet, in spite of all the fine accuracy built into these meters and couples, uncontrollable "cold-end" errors will frequently occur when so-called "compensating" lead wires are used. Here's why . . .

As you know, when wires of different composition are joined together, they form a thermo-electric junction. In the case of "compensating" leads connected to your Chromel-Alumel couples, these junctions are located just outside the furnace and unprotected from heat. Now, as these terminals get hot . . . *and they often get very hot* . . . or when one junction gets hotter than the other, the opposing E.M.F.'s they generate tend to become more and more unequal. And this variable inequality is inevitably reflected by serious plus or minus errors registered by your meter.

How to eliminate this common cause of "cold-end" errors? Simple! Use CHROMEL-ALUMEL EXTENSION LEADS with your CHROMEL-ALUMEL thermocouples. For only by using these wires of identical composition can you be sure of getting the full benefits of accuracy offered by today's improved pyrometers. Our Catalog 59-R tells the complete technical story . . . want a copy?

CHROMEL-ALUMEL couples and leads are available through your instrument manufacturer or pyrometer service company . . . ask for them by name!



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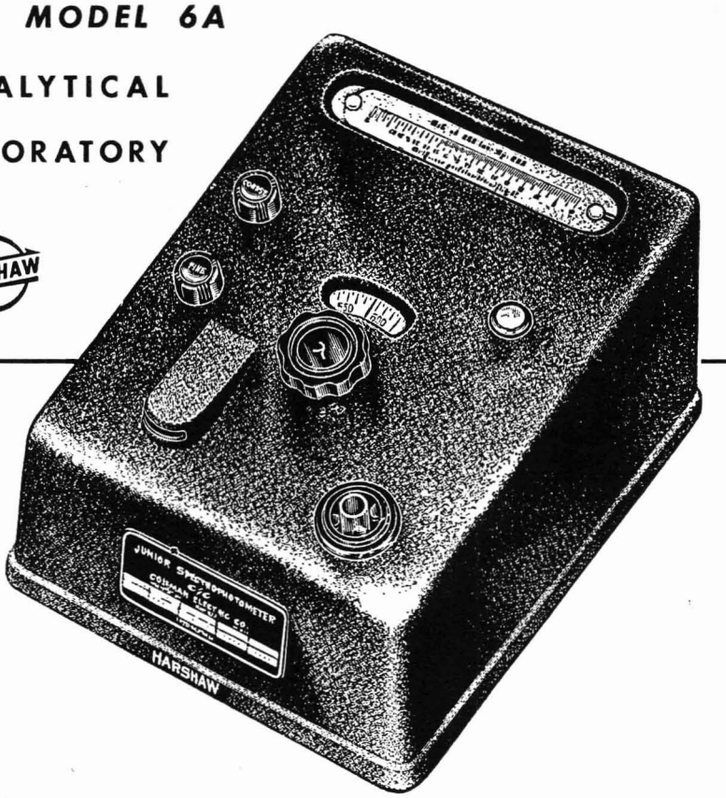

• Whether your work is in metals, foodstuffs, vitamins, or the clinical laboratory, this simplified model of the Universal Spectrophotometer provides simple means of transmission spectrochemistry measurements. Its interchangeable scales are ideal for the analytical laboratory requiring a variety of different analyses from a single instrument. The production laboratory has also found the instruments useful for rapid determinations of copper, manganese, cobalt, nickel, etc. with the speed of the spectrograph but with greater precision and at much less cost.

The Coleman Junior Spectrophotometer fully meets the three requirements necessary for identifying and for measuring solution constituents.

First, it determines the exact color (light wave length) most responsive to the considered constituent.

Second, it is capable of generating light of that exact wave length.

Third, it accurately measures the intensity of that light as affected by the constituent.

For full details write for Bulletin B-211.

H-59540 Coleman Junior Spectrophotometer, complete with Plastic Light Shield, Mounting Pads, Removable Transmittance-Density Scale Panel and Operating Instructions—with **H-59540-15** Storage Battery and Charger with connecting cables **$485.00**

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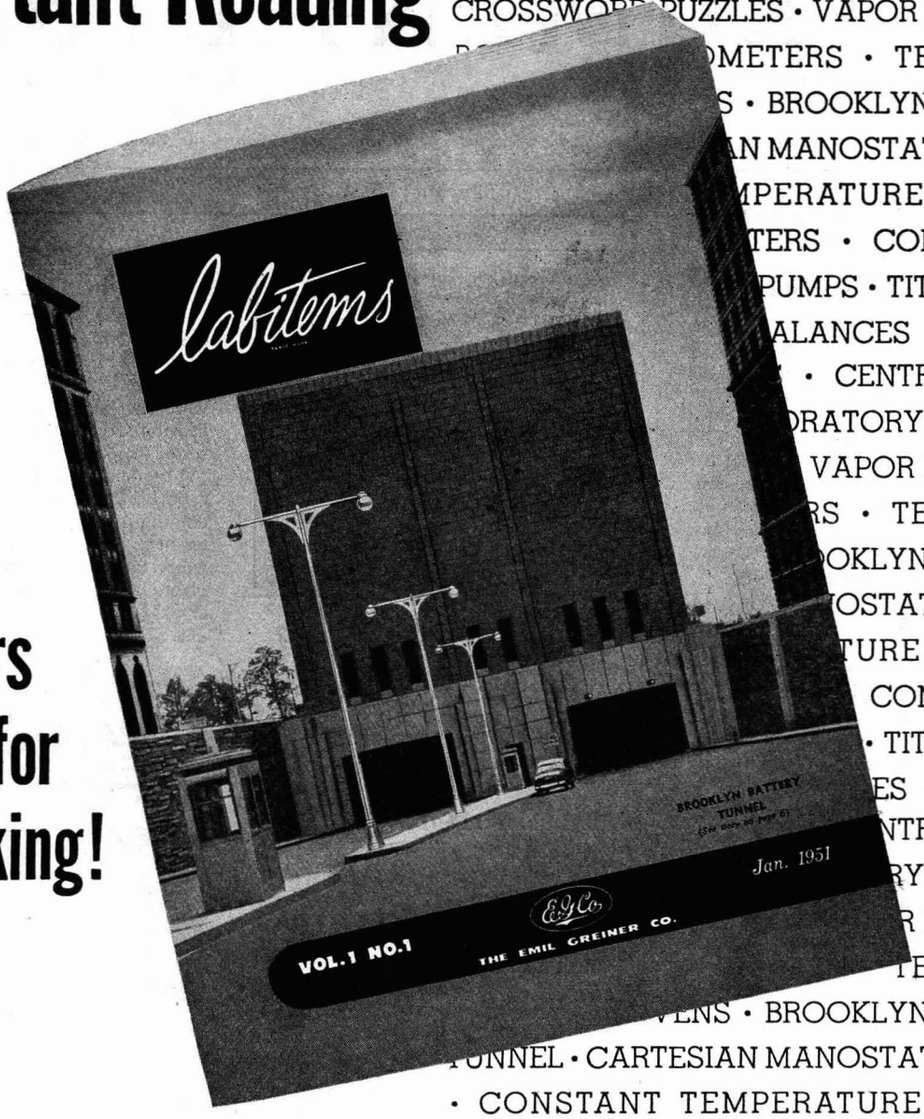

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THE PERKIN-ELMER

INSTRUMENT DIGEST

A condensation of some of the articles appearing in past issues of THE PERKIN-ELMER *INSTRUMENT NEWS*, a quarterly publication of The Perkin-Elmer Corporation, manufacturers of scientific instruments—Infrared Spectrometers, Tiselius Electrophoresis Apparatus, Universal Monochromator, Flame Photometers, Continuous Infrared Analyzer, Low-Level Amplifiers—as well as Astronomical Equipment, Replica Gratings, Thermocouples, Photographic Lenses, Crystal Optics, and Special Instruments for the Government. **For further information, write The Perkin-Elmer Corp., Norwalk, Conn.**

Norwalk, Conn. February, 1951 Vol. 2, No. 4

PIN-TYPE THERMOCOUPLE HAS SPEED, SENSITIVITY

Treatments of the design and performance of existing thermocouples fail to include the advantages and distinctive features of pin-type construction.

The Perkin-Elmer Thermocouple, combining as it does both speed and sensitivity, has many applications other than that for which it was originally designed. The construction and size of the thermocouple may be varied to suit particular applications.

Sensitivity and time constant may be expressed by the following equations:

$$(1)\ E_0 = \alpha\frac{Q}{L} \qquad (2)\ T = \frac{C}{L}$$

where E_0=thermoelectric voltage or sensitivity, α = thermoelectric coefficient of the materials used, Q = the radiant energy per second falling on the receiver, T = time constant, C = heat capacity, and L = energy loss per second per degree C.

It will be seen that a high E_0 depends upon a low L and short T depends upon a large L. The Perkin-Elmer Thermocouple is a compromise in that L is purposely made large due to the pin-type construction, and by selecting materials with a large α (making C as small as possible), the desired characteristics of speed and sensitivity are obtained.

You can receive the complete publication from which these articles were digested.

Write The Perkin-Elmer Corporation, Dept. CEN, Main Avenue (Route 7), Norwalk, Conn., and you will receive regularly THE PERKIN-ELMER INSTRUMENT NEWS, an 8-page quarterly devoted to news of the latest advances in electro-optical instrumentation. Here are some of the features of the current issue:

ELECTROPHORESIS AIDS CANCER RESEARCH
Report on European Developments

ADH SCHMIDT CAMERA IN SOUTH AFRICA
First Photographs Reach U. S.

PIN-TYPE THERMOCOUPLES
Theory, Design and Performance

OSCILLOGRAPHIC INFRARED
Report by Shirleigh Silverman

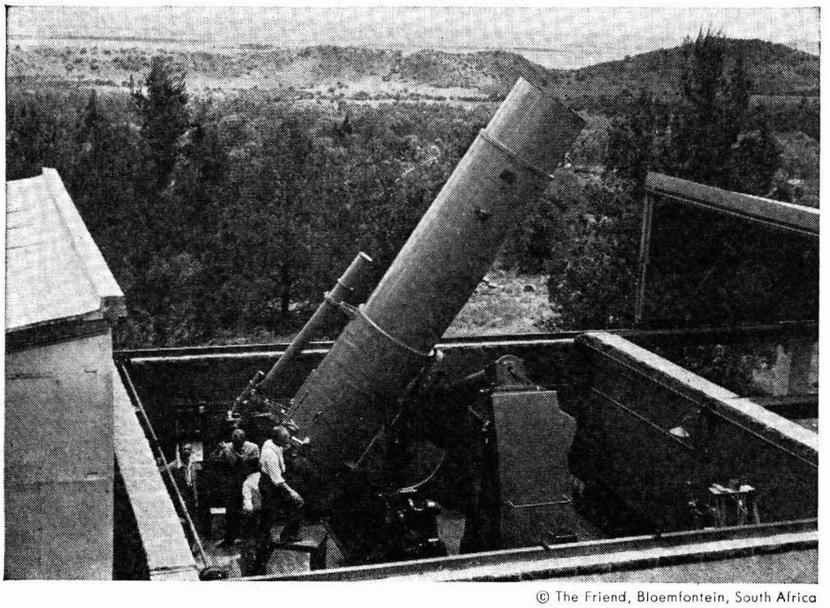
NORWALK PLANT OPENED

Perkin-Elmer has completed moving its plant and office facilities to its new 54,000 square-foot building at Norwalk, Conn. Old friends and new are cordially invited to visit the new plant.

SAFETY SWITCH AVAILABLE

A simple safety switch, designed to prevent damage to the source housing on Model 12 spectrometers, is now available from Perkin-Elmer.

These switches are constructed of a bimetallic strip and microswitch, as reported in INSTRUMENT NEWS, 1, *4*, *5*. They are adjusted to open at approximately 100° C.



© The Friend, Bloemfontein, South Africa

First picture of 32" Baker-Schmidt ADH telescope, made by Perkin-Elmer, used for photographing the Milky Way at Harvard Observatory's Boyden Station, Bloemfontein, S. A.

BOILERWATER CHECKED WITH FLAME PHOTOMETER

Three new 1250 psi Cyclone steam generators at the Dow Chemical Company's Midland, Michigan, power plant have an intake of approximately 4000 gallons of water per minute. Since the water must be silica-free, Dow demineralizes it in a series of ion exchange units.

Frequent sodium determinations with a Perkin-Elmer Flame Photometer on incoming feedwater makeup enable Dow engineers to maintain a tight control over water quality and ion exchange resin capacities. The instrument also serves to locate leakage from mechanical trouble in valves, etc.

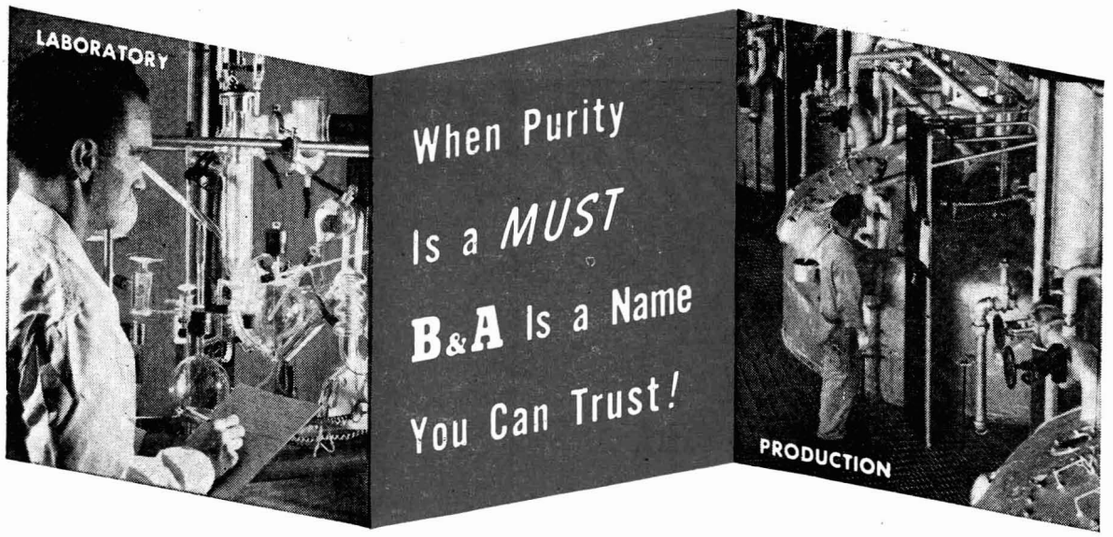
A Perkin-Elmer Flame Photometer in use at the Dow Chemical Company.

ELECTROPHORESIS AIDS IN CANCER RESEARCH

Swiss investigators at the University of Zurich have found marked changes in the electrophoretic serum patterns of rats with chemically induced sarcomas. Total serum proteins were reduced to less than 4 gms per cent, with a marked decrease in albumin; α-globulin was increased to the point where it was sometimes greater than the albumin. English scientists report similar patterns from human patients with various types of cancer.

In Scotland, studies are being carried out on proteins extracted from normal and cancerous tissue. Preliminary results show that virus-induced and chemically-induced fowl sarcomas differ both from each other and from the normal.

A digest of the second of a series of articles on Electrophoresis Developments in Europe by Dr. Dan H. Moore, head of the Electrophoresis Laboratory at Columbia University's College of Physicians and Surgeons.



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the analyst's column

IT IS doubtless true that no conscientious writer of a scientific book ever sends the final proofs to press without wondering how many errors will be found in his work after it appears in unalterable print. This feeling the members of the Committee on Analytical Reagents have shared during recent weeks as they each, separately, read the galleys of "Reagent Chemicals—A.C.S. Specifications" and sent their comments to W. D. Collins for correlation. As one looked over Mr. Collins' shoulder it was interesting to note that whereas most errors were found by several members, and some by all, there were a number detected by only one reader, and that each reader made one or more contributions to these singly-observed corrections. As this is written the page proofs are being read, and one hopes, of course, that this at last will be the error-free book, all the time knowing very well that it will not be. But let this be an invitation to all and sundry to point out the faults. After all, as Alden Emery tells us repeatedly about the Society, this is the Society's book and so it is every member's book.

The forthcoming book of reagent specifications needs the help of all analysts in a way more important than the mere correction of errors. This relates to the real substance of the book—the requirements and the methods of test. How should one go about writing a specification for, let us say, sulfuric acid as a reagent chemical? If it were, instead, sulfuric acid for use in storage batteries, the task would be much easier. In that case, as for any single-purpose chemical, one knows, or at least can find out by experimentation, how much of each probable impurity he is willing to allow, because each will have a measurable effect on the efficiency and life of storage batteries.

If it happens that the desired requirements cannot be realized in commercial production, at least the specification writer knows what kind of compromise he has to make. Not so with reagent chemicals. Most of them are used for many purposes. To one analyst the nitrate content of sulfuric acid may be important, to another the content of reducing matter, and to still another the amount of "heavy metals" it contains. There is only one approach to writing of a specification for a multiuse chemical. That is to describe the closest approach to ideal purity that the reagent manufacturer can attain at a price that the body of users will pay. The qualification is important, but it is not inflexible. The cost of the reagents is so small a fraction of the cost of

(Continued on page 19 A)

THE ANALYST'S COLUMN

analytical operations that a much higher price is much less costly than a half-day lost, a wrong result, or a legal snarl. But the reagent industry enjoys a healthy state of competition, and higher quality, at higher price, is not likely to be forthcoming unless the analyst demands the quality and can persuade his purchasing agent to pay the price. Chemical reagents available in the United States are in all probability the best to be had anywhere in the world, but every good analyst knows, and no reagent producer would deny, that they fall short of ideal purity. Higher quality is possible, but to get it users will have to show they want it and are willing to pay for it.

One thing more, and this relates to the most difficult aspect of preparing specifications for reagent chemicals. How can any one person, or even a group of nine such as the A.C.S. committee, know every impurity that is to be expected in each one of 170 chemicals?

A good reagent specification should prescribe limits for all normal impurities that may be significant in usual analytical work. But how does one know what all the normal impurities are? Sources of raw materials change and so do manufacturing processes.

By way of illustration: In a use of ammonium thioycanate unrelated to analytical work, it was found that this chemical sometimes contains substantial amounts of reducing substances as the result of a faulty manufacturing process. Hence the revised specification for this reagent will contain a requirement for reducing substances.

In reviewing the specification for carbon tetrachloride it became apparent that three of the requirements formerly used are no longer significant, but that because of the common use of this solvent in connection with the dithizone reagent, a new requirement must be added to ensure its suitability for this purpose. In the case of hydrogen peroxide, changes in manufacturing processes have allowed lowering the permissible limit for sulfate from the former value of 0.01 to 0.0005%, thereby greatly increasing the field of usefulness of this reagent. But how can one person, or nine, be sure of being adequately informed on all such matters? Here is another place where analysts can help greatly if they will.

The writer hopes that no analyst will overlook the invitation that has appeared in every publication of the Committee on Analytical Reagents since 1924: "Suggestions for the improvement of the specifications will be welcomed by the committee."

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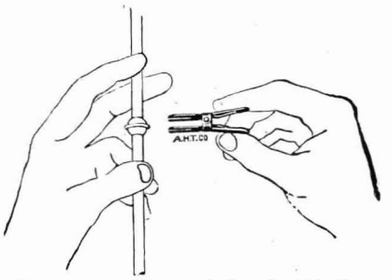

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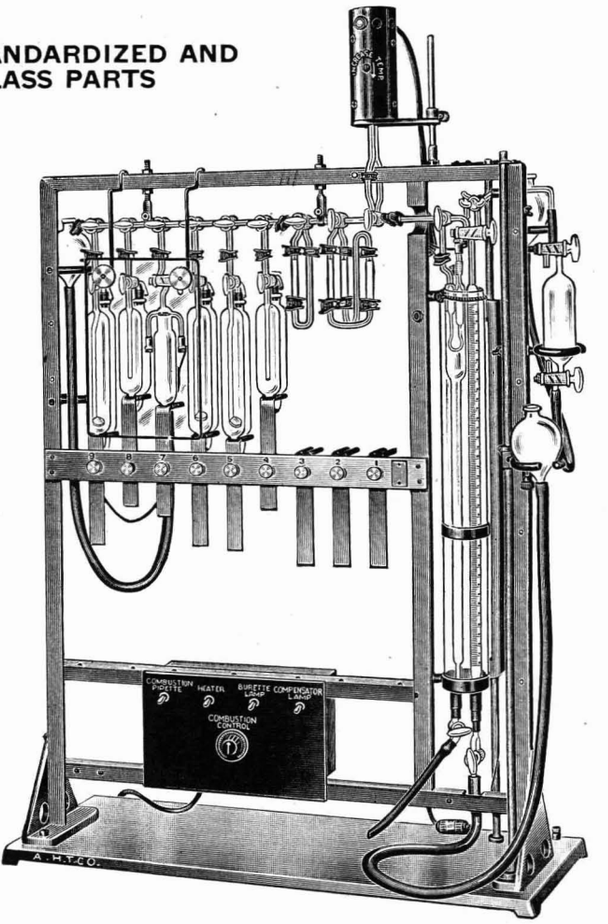

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ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Grass Roots and Instruments

THIS is not the first time that the teaching of analytical chemistry has been discussed editorially on these pages—nor is it likely to be the last. As long as there is an indication of concern, among those who teach the subject and among those who employ the products of such teaching, that education methods are falling short of the desired ends, it will be a part of our responsibility to help them bear the torch which will light up the dark corners and make them available for correction. With the continual and phenomenal advances being made in analysis, it is not probable that a static condition of near perfection in education of analytical chemist-to-be will be arrived at in the foreseeable future.

Four years ago the first of a very successful series of symposia was inaugurated at Louisiana State University. Under the guidance of Philip W. West this annual conclave for consideration of developments in modern analytical methods has maintained the exceptionally high standards set at its beginning. As it has dealt with the most modern methods, a large proportion of its subject matter has been concerned with instrumental analysis. In spite of this, there has been echoed throughout the series the undercurrent "lest we forget," in enthusiasm for our newly found slaves, the fundamentals of analytical chemistry. Dr. West himself delivered a paper at the first meeting in 1948 which dealt entirely with noninstrumental analysis.

The associate editor of this journal, L. T. Hallett, gave at the second symposium an opinion that more emphasis must be placed on the teaching of chemical and physical properties of elements and compounds as related to analytical methods. Your editor, at the 1950 meeting, stated his faith in the belief that, although recognition by analysts of the latent possibilities of instrumentation has brought about a renaissance in analytical chemistry, the classical methods will not be abandoned. However, in the light of increased instrumentation and specialized fields of analysis, care must be taken that the broad fundamentals are not overlooked. The answer lies partially in recognition of the difference between the words "supplant" and "supplement." It is the latter that should characterize the relation of instrumental analysis to conventional methods.

It was inevitable that opinions passed at dinner tables and between session conversations should, after the fashion of the Chicago symposium in 1949, crystallize into a panel on teaching analytical chemistry. The fourth symposium, just finished, closed with such a panel discussion in which 18 professors of analytical chemistry participated. Their major questions were two: (1) What is the status of the teaching of fundamentals? and (2) Where do courses in instrumental analysis best fit?

In spite of the reassurances of the past few years mentioned above, these educators still expressed alarm about the trend away from chemistry. Part of this is due to an increasing nationwide consciousness of gadgets for everything, and it is not surprising that students are attracted more to the use of instruments not only because of time-saving and great potentialities, but also just because they are fun to fool with. Analytical methods have always been and will be devised on the basis of physical and chemical properties of the materials under consideration. Many important advances are being made in noninstrumental methods of analysis (organic reagents, complex ions, catalyzed and induced reactions, spot tests, and chromatography, for example). Although not so glamorous as instrumental analysis, they must not be overlooked. To accomplish this better it was the unanimous opinion of the group that more inorganic chemistry must be taught at the lower levels and that qualitative analysis courses offered perhaps the best opportunities for teaching chemical reactions and chemical principles. Two points of definite agreement emerged from the discussion—qualitative analysis should be a unit course, preferably taught by analytical faculty; closer integration with quantitative analysis should be developed.

The student cannot wait until his entrance into industry to learn the use of instruments. Where, then, does such a course best fit? That such a course is imperative for graduate training was agreed. Because training in the care and use of instruments is, in this day, a fundamental part of a chemist's education, even the student getting only a bachelor's degree should become aware of advantages and disadvantages of instrumental methods; hence the consensus of opinion on the desirability of a graduate-senior level course. Many students in the beginning courses can pick up later the limited knowledge of instruments they will need. Therefore these courses should be restricted to fundamentals of classical qualitative and quantitative analysis, leaving instruments for the senior year and the serious student. Advantages cited for this undergraduate work were an acquaintance for the student of the real scope and organization of analytical chemistry as it now exists, a foundation for a theory course at the graduate level, and a method of creating interest and attracting good students into advanced studies.

Disadvantages of a graduate-senior level course were just as carefully considered by the group. For the small college particularly the cost of instruments is a serious obstacle. This is not critical, however, since many methods and principles can be taught with improvised equipment, and demonstrations can be used effectively. Crowded curricula offer difficulties, but an elective in instrumental analysis may have as much merit as a course in advanced organic or physical chemistry. The final disadvantage is a possibility of loss of interest in classical methods. This—and all the rest of the success of a course in analytical chemistry—depends to a great extent upon the instructor. Whatever fears we may have about trends in analytical instruction, whatever conclusions we may reach for its improvement are so fundamentally related to the nature of the instruction received that only the faculty members themselves can provide the final answer to the success of any program.

3rd Annual

Review of Analytical Chemistry

CONTINUING our review of developments in analytical chemistry, the following 8 articles report progress in important fields of application. The fundamental developments were reviewed in our January issue. A combined reprint of the reviews from both issues is available at $1.50 per copy from the reprint department of the AMERICAN CHEMICAL SOCIETY.

—The Editors

COATINGS

T. G. ROCHOW AND R. W. STAFFORD, *American Cyanamid Co., Stamford, Conn.*

THE analysis of coatings is the concern of this review. It follows the plan of the past two years, and covers organic high polymers and their associated oils, pigments, and solvents. The term "coating" is construed to imply that the material has been applied or otherwise processed, and "dried." The scope of the review is abridged by the arbitrary omission of inorganic coatings such as vitreous enamels, metals, and chemically treated metals, and by disregarding what are considered to be the less common uses.

General schemes for the analyses of organic coatings are considered first. The next section is devoted to resinography, which, although of general applicability, was considered to be of sufficient fundamental and potential importance to justify separate consideration in some detail. Monographs on the analysis of separate parts of organic coatings are then taken up in the order: separate classes of resins, relevant oils, pigments, and specific constituents or functional groups. Auxiliary references to a limited bibliography on relevant descriptive and evaluative literature are presented as general background information.

GENERAL ANALYTICAL SCHEMES FOR ORGANIC COATING

A number of investigators have reported on general procedures directly or potentially applicable to the identification of high polymeric constituents of organic coatings.

Estartus (*15*) suggests a systematic scheme, based on known tests, for the identification of the main classes, which are determined by the presence or absence of elements other than carbon, hydrogen, and oxygen. Pallaud (*51*) divides plastics into two large groups, and bases qualitative identification primarily on physical tests such as density, fluorescence, and x-ray methods, with considerable emphasis also on determination of saponification value, dry distillation, and elementary analysis. Monterumici and Parrotta (*43*) outline a scheme for the identification of thermoplastic synthetic resins which employs saponification values, nitrogen or halogen content, heat depolymerization, the Storch-Morawski test, and other color or precipitation tests. Fitzgerald-Lee (*17*) identifies plastics by the odor and the appearance of the flame resulting from the action of a Bunsen flame on a small strip of the plastic in question.

Considerable work has also been done in the interesting solubility field of high polymer separation. Nitsche and Toeldte (*49*) use the determination of solubility as a means for identification and characterization. Desreux (*11*) describes an automatic apparatus for the micro- or macrofractionation of polymers, using continuously varying solvent mixtures, and packed columns of polymer precipitated on Celite or fine sand. Claesson (*9*) discusses the difficulties involved in the chromatographic separation of materials of high molecular weight and reports data on molecular weight distribution, in good agreement with those resulting from other methods, obtained on very dilute solutions of methyl methacrylate and polyvinyl acetate in acetone. De Brouckere, Bidaine, and van der Heyden (*8*) suggest the use of a counterflow method to facilitate the fractional precipitation of pure high polymers.

In the field of general properties, Goss (*23*) presents reference tables of properties of thermosetting molding, thermoplastic molding plastics, and thermosetting plastic laminates.

RESINOGRAPHY AND DEPICTION OF MACROMOLECULES

During the past year, there was much development of electron microscopical techniques for the depiction of the fundamental resin units—i.e., the macromolecules or small aggregates thereof. Siegel, Johnson, and Mark (*64*) spray very dilute solutions of polystyrene in a "poor" solvent, cyclohexane, onto a collodion substrate. By electron micrography of the shadow-cast specimens, the molecules of polystyrene are manifested as prolate spheres whose diameters are used together with the bulk density and, presumably, Avogadro's number, to calculate the number-average molecular weight which, for four molecular size classifications, agreed with the molecular weight as calculated from the intrinsic viscosity. Three limitations are concluded to be: practical resolving power of the microscope and the replicas, interference from particulate structure of the supporting substrate, and precision of measurement (±25 A.). The lower measurable limit of molecular weight is estimated to be 0.5×10^6. The accuracy is concluded to increase rapidly with molecular weight. Kaye (*32*) prefers an alloy (2 aluminum–3 beryllium) to a high polymer as either a supporting or replicating medium because the alloy possesses high strength and low scattering. An electron micrograph of the blank metallic substrate shadowed with another alloy (4 platinum–1 palladium) shows that the substrate is composed of particles in the order of 100 A. in diameter. Assuming a unit density, the present authors calculate a molecular weight in the order of 320,000. Such coarse structure would definitely interfere with the manifestation of macromolecules by most high polymers intended to be used as coating materials. To show coarser structures, however, the aluminum-beryllium alloy suggests a whole realm of metallic media as electron microscopical replicas or supports. As a recent development, Kaye and Peck (*33*) describe an etch technique to reveal internal structure. For example, cellulose acetate is etched with cold (35° to 40° C.) acetone and the reaction is stopped arbitrarily with cold ethyl al-

cohol. The principle of controlled etching appears to be another important contribution to resinography by Kaye and his co-workers.

Morehead (*44*) correlates the extents of resolution obtained by various applications for microscopy to films and fibers, employing "useful" magnifications from 10× to 100,000×. Regarding the latter magnification, electron micrographs are shown of the "skin" surface and "core" surface of a cellulose film regenerated from a viscose sol on glass by a standard yarn-spinning bath. The skin (spin-bath surface) stains with Victoria Blue B and manifests particle sizes which the present authors, using density equal to 1.5, calculate to be from approximately 200,000 to 1,700,000; average, 540,000. The core (glass surface) does not stain with Victoria Blue B and manifests particles calculated to have molecular weights from 960,000 to 7,500,000; average 4,200,000. Newman (*48*) shows the particulate structure of the cellulose regenerated from a cuprammonium sol, by the electron micrography of a microtomed section of the fiber. Assuming a density of 1.5, the present authors calculate the macromolecular weights to be from 290,000 to 20,000,000 with an average of 820,000.

Rochow and Rochow (*59*) use the fracture technique (*60*) to expose the internal structure of extensomers—for example, natural rubber and an extensible silicone. Electron micrographs show the distribution of the particles of channel black in the vulcanized rubber and of titania in the cured silicone. At a magnification of 100,000, macromolecules of the extensomers are depicted. Their outlines are fuzzy, but they enable some differentiation of size and shape. Using bulk specific gravities and Avogadro's number, the particle diameters in rubber correspond to molecular weights of 8000 to 2,200,000, with most around 650,000. Using the silicone extensomer as an example, it is demonstrated that there is practically no difference between calculations based on bulk specific gravity and on molecular volume of the monomer. Molecular weights of the depicted silicone macromolecules vary from 310,000 to 2,500,000, with most at 610,000. These values agreed almost exactly with those which Scott (*62*) calculates from osmotic pressures of precipitated size fractions. The resinographic depiction of macromolecules in situ may suggest the pictorial study of a single fracture surface during curing, aging, and other influences on molecular size and shape.

SPECIFIC CLASSES OF HIGH POLYMERIC MATERIALS

Haslam and Newlands (*25*) separate the polymeric fraction of polyvinyl chloride plastics by centrifuging a tetrahydrofuran solution, precipitate the polymer with ethyl alcohol, and analyze by chlorine determination and infrared spectral analyses. Tribot and Simon (*69*) effect the practically complete evolution of hydrogen chloride from polyvinyl chloride at temperatures above 100° C., and determine the halogen argentometrically. Gate, Mayne, and Warson (*21*) propose that partially hydrolyzed polyvinyl acetate polymers be designated by the easily determined saponification values, which are readily calculable to hydroxyl values. Haslam and Soppet (*27*) list analytical methods for methyl methacrylate polymers, and go into some detail on vacuum depolymerization. The depolymerization products are identified chemically and by infrared spectral analysis. Pektor (*53*) discusses the qualitative analysis of vinyl-type synthetic resins used in leather finishes, including characteristic fluorescence and changes on fusion. Brockway (*7*) lists a rapid qualitative test for acrylonitrile by preparation of the picrate of β-piperidinopropionitrile, formed by the action of piperidine on the acrylonitrile.

Krajcinovic (*35*) detects free carboxyl groups in cellulose materials by the formation of an addition compound with benzidine, followed by diazotization and coupling with 2-naphthol to yield a characteristic color. Neu (*46*) differentiates between cellulose ethers and cellulose ether glycolic acids by the precipitate formed when dimethylalkylbenzylammonium chloride (Zephirol) reacts with the latter. Manneck (*39*) reviews the detection and determination of water-soluble cellulose ethers. Genung (*22*) discusses the analysis of cellulose derivatives under three headings: esters for acyl content by saponification and distillation; mixed esters by partition, extraction, or determination of characteristic functional groups; and ethers. Devor (*12*) states that the Molisch test is improved by using random presulfonated 1-naphthol.

Kappelmeier and van Goor (*31*) report on the complete analysis of alkyd resins. In the absence of phthalic, adipic acid can be easily determined as the potassium salt, using the Kappelmeier phthalic method and determining the melting point of the acid before and after recrystallization to confirm its identity. Interference by methyl adipic acid is detected by determining the potassium content of the salt or by converting the salt into the ethyl ester, and determining the saponification value of the ester. Succinic, sebacic, and tetrachlorophthalic acids can also be determined as the salts. Details are given for the qualitative separation of phthalic from other dibasic acids. Monocarboxylic acids are separated and identified in the usual way. The alcohols, after removal of possible ether-soluble components, are separated quantitatively and identified further by hydroxyl values. Aguadisch and Sachs (*1*) precipitate dipotassium phthalate in the usual way, absorb the fatty acids on paraffin wax prior to gravimetric determination, and measure glycerol iodometrically after oxidation by potassium dichromate. Novak (*50*), after saponification of phthalic alkyds, separates the phthalic and aliphatic acids by partition between water and ether and titrates each layer.

Donnally (*13*) hydrolyzes methylol ureas to urea plus formaldehyde by the action of 0.3 *N* neutral sodium phosphate solution, and proposes an iodine method for the determination of the liberated formaldehyde. Figarat (*16*) saponifies the nonvolatile fraction of varnishes, treats the products with petroleum ether, and dry distills the undissolved phenolic fraction, using color tests to identify the phenol.

Manalo and West (*38*) report on the analysis and composition of manila elemi. Kamath and Mainkar (*30*) suggest an iodometric method for the determination of the acid value of lac.

OILS

Mehlenbacher and associates (*41*) have issued the report of the American Oil Chemists' Society Committee on the Analysis of Commercial Fats and Oils. The report includes those of subcommittees on color standards, analysis of drying oils, and determination of thiocyanogen values. Linder and Persson (*37*) modify the Wolff procedure for the separation of fatty and resin acids through selective esterification by replacing the sulfuric acid with benzene sulfonic acid and by using butyl alcohol and benzene as saponification solution. The water of condensation is trapped off in a specially designed apparatus, forcing the esterification of the fatty acids to completion. Pohle and Mehlenbacher (*55*) modify the periodic acid method for free and combined glycerol by removing the former by water extraction of a chloroform solution of the sample prior to testing with periodic acid. Hezel (*28*) has developed a new procedure for the determination of saponification value, using thymolphthalein and bromophenol blue as indicators. Metallic soaps can be determined separately, and the reacted and unreacted maleic acid of maleinated adducts can also be determined by the method. Zöllner (*71*) applies the Hezel method, to determine the saponification value of waxes. Benham and Klee (*3, 34*) apply a modified Rosenmund-Kuhnhenn method to the determination of the iodine number of unconjugated fats and oils, and by greatly extending the reaction time, make the method applicable to oils containing conjugated double bond systems.

PIGMENTS

Potts (*57*) has reported on the polarographic determination of titanium in paint pigments, and Augusti (*2*) has discussed in con-

siderable detail the microchemical differentiation and recognition of mineral colors and of anions and cations in mineral pigments. Tanke (*67*) reports on the chemical and physical properties and tests of the ultramarine colors. Davidson (*10*) shows with electron micrographs how size, shape, and size distribution influence the practical characteristics of pigments. He illustrates variations in tinting strength with pigment size and shape, and pleochroism and color tone with size, and change in size with time in an effective medium. He points out that the sizes and shapes of the ultimate particles may be much different from those of the aggregates existing in the paint prepared for application. He does not include the possibilities of further change after application and drying. Vallaud (*70*) presents a review and a discussion of various accepted methods for the determination of aromatic hydrocarbons in solvents and, on a general basis, in solutions, paints, varnishes, cements, etc. Schaefer (*61*) lists color reactions for some accelerators in common use.

SPECIFIC CONSTITUENTS

This section includes separate constituents of coating materials.

Eisenberg and Hillig (*14*) identify succinic acid microscopically as barium succinate. Rauscher and MacPeek (*58*) identify the esters of monobasic acids by the use of ethanolamine to evolve the alcohol and yield the characteristic *N*-β-hydroxylethyl amide. Paschke and Wheeler (*52*) report on the preparation and melting points of the dihydrazides of dicarboxylic acids from glutaric to sebacic. Swern, Knight, Shreve, and Heether (*66*) find the infrared spectrophotometric method to be more rapid, specific, and accurate than the lead salt–alcohol method for the determination of *trans*-octadecanoic acids and esters. Marvel and Rands (*40*) describe procedures for the separation of water-soluble organic acids by partition chromatography. Peters and Clark (*54*) determine the composition of commercial stearic and palmitic acids by the use of curves of composition versus titer, adding a known amount of either acid if the titer should fall on a eutectic or flat part of the curve.

Tourliere (*68*) determines the concentration of primary alcohols and their mixtures by distillation, against standards, in chromic oxide solution. Hough (*29*) applies paper partition chromatography to the separation of polyhydric alcohols. Neu (*47*) determines the limits for the detection of glycols in glycerol by the Kröller (*36*) method. Porter and Reid (*56*) determine the concentration of di- and triethylene glycol in solutions containing 1 to 20% water from the viscosity at 30° C. Middleton and Stucky (*42*) determine the purity of propylene glycol by the measurement of ether points. Stempel (*65*) converts glycerol to quinoline by condensation with aniline and detects the quinoline by the white precipitate it yields with potassium mercuric iodide. Habicht (*24*) reviews the acetin, iodide, and dichromate methods for glycerol. Francis (*18*) determines the diglycols in mixtures of ethylene and propylene glycols by prior oxidation of the latter with periodic acid. After distillation of the aldehydes, the diglycols are oxidized with potassium dichromate, and are measured polarographically before and after oxidation. Shaffer, Critchfield, and Nair (*63*) report on the chemical and physical examination of some polyethylene glycols. Haslam and Ruddle (*26*) determine small amounts of tetrahydrofurfuryl alcohol by treatment with standard ceric sulfate solution, followed by titration of the excess with Mohr's salt.

Fungairino (*20*) reviews the gravimetric, volumetric, and colorimetric methods for the determination of furfural. Berton (*4*) reports on the analysis of furfuraldehyde by the ultraviolet absorption spectrum of its vapor. Bremanis (*5*) gives the details of the photometric determination of formaldehyde with chromotropic acid, while Bricker and Vail (*6*) report on the microdetermination of formaldehyde with the same reagent, determining spectrophotometrically the optical density of the dye formed versus concentration.

Nametkin and co-workers (*45*) identify phenols as the amides and anilides of phenoxyacetic acid and list melting point data for 154 phenols. • Friedel, Pierce, and McGovern (*19*) effect the qualitative and quantitative analysis of phenol, cresols, xylenols, and ethylphenols by inspection of the infrared spectra in the 8 to 10μ region.

ACKNOWLEDGMENT

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Essential Oils and Related Products

ERNEST GUENTHER AND EDWARD E. LANGENAU
Fritzsche Brothers, Inc., New York, N. Y.

THIS annual review of analytical procedures for essential oils and related products follows the general outline established in the two previous reviews (*22*, *23*).

Several important publications of interest to the essential oil chemist have appeared and are discussed below in some detail. During the year very little original work has been reported which deals directly with the analysis of essential oils.

OFFICIAL COMPENDIA

The fourteenth revision of the Pharmacopeia of the United States (*69*) was published during the year and became official on November 1, 1950. Monographs are included for the following products of interest to the essential oil trade:

Oil of eucalyptus, oil of cade (juniper tar), and thymol, which formerly appeared in the thirteenth revision, have been transferred to the latest edition of the National Formulary. A new monograph for dimethyl phthalate has been added. Oil of cedar leaves has been deleted; this oil had been introduced as a wartime substitute for the imported lavender oil in preparing official tincture of green soap.

Because of the increased importance of propylene glycol as a solvent, this item has been transferred from the National Formulary to the United States Pharmacopeia. The cumbersome assay for glycol content which appeared in the N.F. VIII (*42*) has been omitted; however, the close limits established for the physical properties (especially for the specific gravity) seem adequate to assure a high glycol content.

Following the principle already established by the National Formulary, the United States Pharmacopeia now makes use of a new subheading in the monographs for essential oils, "Solubility in —% alcohol"; this is to indicate that such data for solubility in dilute alcohol represent specifications for purity and are not to be confused with the supplementary informative statements which appear under the subheading "Solubility."

In the monograph for peppermint oil, the color test of the eleventh revision (*67*) is readopted to assure the absence of adulteration with oil of *Mentha arvensis*. Such color tests generally are unsatisfactory for essential oils; however, the present test is preferable to the furfural test of the thirteenth revision (*68*).

Several minor modifications have been made in order to improve certain tests and assays, and to bring specifications of the physical properties within accepted limits.

The ninth edition of the National Formulary (*43*), which also became official on November 1, 1950, includes monographs for the following items:

Balsams, etc.	Page
Orris	365
Rosin	437
Tar, juniper	533
Tar, pine	534
Turpentine	558

Allyl isothiocyanate (volatile oil of mustard, natural and synthetic) has been deleted from this latest edition.

For the assay of benzaldehyde, bitter almond oil, and cinnamic aldehyde, the hydroxylamine hydrochloride method has been substituted for the Stillman-Reed method of the eighth edition. The new assays, which are similar to those of the seventh edition, give more satisfactory results if the end points are to be determined visually.

NEW TEXTS AND PUBLICATIONS

The fourth volume of Guenther's "The Essential Oils" (*21*), which appeared in October 1950, continues the series of monographs on the individual essential oils which was begun in the third volume. The chapter dealing with camphor oil was prepared by the eminent authority, Teikichi Hiraizumi, and represents the first complete report on the camphor industry to appear in western literature. Penfold and Morrison are the authors of the monographs on the individual eucalyptus oils. Reliable analytical data are presented for the commercially important oils of the following botanical families: *Gramineae*, *Lauraceae*, *Burseraceae*, *Myrtaceae*, *Umbelliferae*, and *Geraniaceae*.

The Scientific Committee of the Essential Oil Association of the United States has continued its program of establishing specifications for the best grade, commercially available, of essential oils and related products. Thirteen new "Specifications and Standards" (*13*) have been prepared for submission in December 1950 to the membership of the association for final approval. These include the following:

- Essential oils
  - Oil of cinnamon leaf
  - Oil of clove leaf
  - Oil of dill weed, American
  - Oil of geranium, Algerian
  - Oil of geranium, Réunion
  - Oil of fir needles, Siberian
- Synthetics and isolates
  - Aldehyde C-12, lauric
  - Aldehyde C-12, M N A (methyl nonyl acetaldehyde)
  - Amyl cinnamic aldehyde
  - Linalool (from oil of bois de rose, Brazilian)
  - Methyl phenyl carbinol (styralyl alcohol)
  - Methyl phenyl carbinyl acetate (styralyl acetate)
  - Phenyl propyl alcohol
- Gums, etc.
  - Basic analytical procedures and tests for gums

In keeping with the general trend, the specifications for specific gravities are now given at 25°/25°. However, an individual factor is reported for each oil, synthetic, or isolate which permits conversion of the specific gravity from 25°/25° to 15°/15°.

In addition, several new "determinations" have been proposed:

E. O. A. No. 1-I. Hydroxylamine Hydrochloride Method for Aldehydes
E. O. A. No. 1-J. Neutral Sulfite Method for Aldehydes and Ketones
E. O. A. No. 1-K. Determination of Phenols
E. O. A. No. 1-L. Determination of Heavy Metals

During the year, a new British trade publication appeared, *The International Perfumer* (*27*); Volume I, No. 1, was released in August.

Samuel P. Sadtler and Sons, Inc. (*56*), have prepared infrared spectral absorption charts for over 1000 pure chemical compounds, many of which are used by the essential oils industry. Reproductions of the charts, showing per cent transmittance against wave length in microns and wave number, are available.

The *Berichte von Schimmel & Co.*, 1948 (*57*), covering the years 1944 to 1947, has been reviewed briefly in the *Perfumery and Essential Oil Record* (*1*).

ANALYTICAL PROCEDURES FROM SCIENTIFIC AND TECHNICAL LITERATURE

Acids. Marvel and Rands (*37*) described a procedure for the separation of water-soluble organic acids by partition chromatography; using their techniques, such chemically similar acids as *o*-, *m*-, and *p*-hydroxybenzoic acids can be separated. Nijkamp (*48*) reported a modification of Elsden's (*11*) chromatographic method which permits the determination of acetic acid.

Matthews, Warren, and Michell (*38*) presented considerable data relating to the x-ray powder diffraction patterns of the silver salts and certain amides of the fatty acids. Grabar and McCrone (*19*) presented detailed crystallographic data for phenylacetic acid.

Alcohols and Phenols. Lintner, Schleif, and Higuchi (*32*) applied an electrometric titration technique employing lithium aluminum hydride to the determination of alcohols; the reduction potential of the solution remains high until the end point is reached, when a slight excess of the alcohol causes a marked drop in potential. Much exploratory work is being carried out by these workers and their colleagues at the University of Wisconsin regarding the application of such a method to the analysis of essential oils. Tetrahydrofuran appears to be an excellent solvent for this determination. The use of *p*-aminoazobenzene has been suggested as a possible chemical indicator to replace the electrical end point, thus making the method more attractive for a routine analytical procedure. In a supplementary note, Higuchi (*25*) pointed out that the oxygen of the air affects adversely this determination; it is recommended that all such analyses be run under nitrogen and that the hydride solution be stored under this inert gas.

Danielsson (*10*) made a critical study of the determination of menthol in peppermint oil, comparing the official method of the Swedish Pharmacopeia with that of the United States Pharmacopeia; from his report, it would appear that the latter method is to be preferred.

For the identification of phenols, Valentin (*70*) reported on a method for the determination of the hydroxyl group using acetic anhydride in dioxane and fluoboric acid as a catalyst; the acid produced is titrated with 1 *N* sodium hydroxide. Water will interfere with this determination. It would appear that the catalyst might cause dehydration of several of the sensitive compounds found in essential oils, thus limiting the use of this method.

Pickthall (*52*) presented analytical data on those carbinols (and their esters) which are of importance to perfumery. Because much of this information is not available in the literature, the physical and chemical data should prove of value. However, the analyses were made with commercial samples, and not with highly purified chemical material.

Nametkin *et al.* (*41*) presented data on the amides and anilides of the corresponding phenoxyacetic acids of some 150 phenolic compounds.

Esters. Rauscher and MacPeek (*53*) described a procedure for the identification of esters of monobasic acids by treatment with ethanolamine; the free alcohols are distilled (or extracted) and identified by conventional methods; if possible, the acids are separated directly as the solid *N*-β-hydroxyethylamides and identified by melting point. Formates and certain aliphatic hydroxy acids cannot readily be identified by this procedure.

Hampton and Newell (*24*) presented infrared spectroscopic data for nineteen esters; the maximum absorption frequency for ester carbonyl occurs at 1740 cm.$^{-1}$ The effect of a halogen

on the α-carbon and of conjugation with the —C=O group causes a shift in this maximum. Application of the method to quantitative analysis is suggested. Buckles and Thelen (*6*) reported on the scope and limitation of the hydroxamic acid test for the detection of the ester carbonyl group; acids, phenols, aldehydes, and other groups were studied and a qualitative procedure was suggested for distinguishing between esters and these other classes.

The fluorescence of 98 coumarin derivatives as a function of pH was studied by Goodwin and Kavanagh (*17*).

Aldehydes and Ketones. Mitchell and Smith (*40*) proposed an analytical procedure for the determination of aldehydes in the presence of ketones; their method is based on an oxidation of the aldehyde with silver oxide in an aqueous medium. Sodium hydroxide is added, and the excess is back-titrated with standardized hydrochloric acid. Under these conditions, ketones do not react (with the exception of cyclohexanone). This technique can be modified to permit determination of acids, esters, and alcohols in the presence of aldehydes. Such a method should prove of value in the analysis of essential oils for the differentiation of the aldehyde and ketone carbonyl function.

Trozzolo and Lieber (*66*) suggested the use of the "hydroxylamine number," the number of milligrams of potassium hydroxide which is equivalent to the hydroxylamine required to oximate the carbonyl function in 1 gram of sample. The need for such a number frequently has been felt by essential oil chemists, especially in those cases where the carbonyl components of an oil are not fully known. Unfortunately, in the past, the spasmodic attempts to introduce its use for essential oils have not been successful, primarily because of the lack of a firmly established procedure for oximation that is satisfactory and adequate for all aldehydes and ketones. Smith and Mitchell (*60*) suggest a further modification of the hydroxylamine hydrochloride method for the determination of carbonyl compounds in the presence of organic acids; they point out the well-known fact that organic acids do not interfere in the determination if bromophenol blue is used as an indicator. Another modification of the same method for the estimation of aldehydes, ketones, and acetals has been suggested by Maltby and Primavesi (*36*); no advantage is offered by this proposed modification.

Gerber, Kuznetsova, and Nelman (*16*) presented data for a polarographic method of determining aldehydes and ketones with conjugated bonds; the carbonyl compounds studied included citral, cinnamaldehyde, and several others of interest to the essential oil chemist. Korshunov, Kuznetsova, Sazanova, and Kirillova (*30*) continued this study, applying the method to aromatic ketones and aldehydes.

For the detection, identification, and separation of aromatic aldehydes in mixtures with other carbonyl compounds, Spasov and Ivanov (*63*) recommended treatment with chloral hydrate and ammonia; most aliphatic and aromatic ketones do not react, and most aliphatic aldehydes yield noncrystalline compounds under the prescribed conditions. Such a method would appear to have only limited use for application to essential oils. Fungairiño (*15*) reviewed the methods for the determination of furfural; gravimetric, volumetric, and colorimetric procedures are discussed. McCrone (*35*) presented crystallographic data for vanillin; two polymorphic forms are obtained when crystallization is carried out from chloroform–carbon tetrachloride solutions; data are given for form I, which is present in all normal recrystallizations from this solvent pair.

Seidel, Schinz, and Ruzicka (*58*) maintained that ozonization is useful for the determination of the semicyclic methylene group in irone and the ionone series; the validity of their technique had previously been questioned by Naves (*44*, *45*). Ruzicka *et al.* do not claim high precision for the method, and suggest that a correction factor should be applied. In a later paper, Naves (*46*) again contested the validity of the technique employed.

Günthard, Ruzicka, Schinz, and Seidel (*20*) questioned the value of the Raman spectra for distinguishing between the α, β, and γ forms of irone; they prefer to use infrared extinction coefficients at 812 cm.$^{-1}$ and 890 cm.$^{-1}$ for estimating the relative amounts of γ- and α-irone.

Determination of Water. Roberts and Levin (*55*) proposed a method employing both azeotropic distillation with benzene and the subsequent use of the Karl Fischer reagent; it is claimed that this method can determine as little as 0.0002% of water in oils.

Baker and MacNevin (*5*) suggested the use of lithium aluminum hydride as a reagent for the determination of water. The sample must contain no functional group that might react with the reagent; this greatly limits its usefulness for essential oils.

Ricciuti and Willits (*54*) published a report on the Karl Fischer reagent; the preparation of the reagent is discussed and a method of standardization proposed which avoids the preparation of absolutely anhydrous methyl alcohol, and the difficulties inherent in weighing out small amounts of water.

Miscellaneous. Melardi (*39*) investigated the resorcinol method for the determination of cineole in eucalyptus oils; he reported that by increasing the strength of the resorcinol solution from 50 to 60%, cineole losses were kept at a minimum.

Ishler, Borker, and Gerber (*29*) described a procedure for the determination of safrole in soaps. The soap is first precipitated with silver nitrate; the safrole is separated by steam distillation and then measured by ultraviolet absorption at 285 mμ. Littlejohn (*33*) reviewed the methods for the estimation of safrole in sassafras oils and described in detail an apparatus and procedure for a cryoscopic method. de Souza (*62*) published a review dealing with Brazilian sassafras oil which included a bibliography for suitable analytical methods.

Ogg and Cooper (*49*) described a technique for the determination of unsaturation by microhydrogenation.

Fritz (*14*) recommended a solution of perchloric acid in acetic acid for titration of organic bases in nonaqueous solutions; methyl violet can be used as indicator, or a pH meter using glass-silver electrodes.

Naves (*47*) published a general discussion dealing with the quality control of perfumery materials. In this paper, he stressed the importance of analytical control.

Several papers dealing with the determination of functionality in organic compounds were published early this year; these were originally presented before the Division of Analytical and Micro Chemistry at the 116th meeting of the AMERICAN CHEMICAL SOCIETY in 1949. Introductory remarks were made by Elving (*12*). Siggia (*59*) discussed the determination of the organic functional groups by chemical means; Coggeshall (*8*), by molecular spectroscopy; Lykken (*34*), by electrical measurements. These important papers should be studied carefully by all organic analytical chemists. Much basic material of great value to the analyst is presented. Determinations based on functionality have been much employed in the essential oil industry.

Higuchi, Lintner, and Schleif (*26*) discuss the electrometric titration of functional groups using lithium aluminum hydride solution. Lieb and Schöniger (*31*) also suggested the use of lithium aluminum hydride for the determination of functional groups. In place of the electrometric titration proposed by the workers at the University of Wisconsin, Lieb and Schöniger make use of the apparatus of Soltys (*61*) for a microdetermination involving measurement of the liberated hydrogen. The procedure is a modification of the Zerewitinoff technique.

Wilson (*71*) presented a report on flavors and nonalcoholic beverages. Analytical data showed that the official method for the determination of alcohol in citrus extracts (*2*) is also applicable to extracts of almond, clove, cinnamon, peppermint, spearmint, and wintergreen. The tentative methods (*3*, *4*) employed for the determination of essential oil content in extracts proved adequate.

Cartwright (*7*) discussed briefly organoleptic panel testing.

In a continuation of their series on analysis, Patin and Vignau (*51*) dealt with the evaluation of perfume products obtained by extraction with solvents. The separation of alcohols from essential oils was also reviewed by these writers.

Adulteration. Otto and Siering (*50*) called attention to the fact that the standard test for distinguishing between Sumatra and Siam gum benzoin is not reliable; this test is based on oxidation of cinnamic acid to benzaldehyde. The Sumatra variety of gum contains cinnamic acid; the Siam variety reputedly contains none. Their experiments indicated that a genuine sample of the Siam gum actually contained about 3% of cinnamic acid.

Goswami (*18*) briefly discussed methods for detecting adulteration in essential oils.

Stempel (*65*) suggested a test for the detection of glycerol by condensation with aniline to quinoline; the quinoline is determined by the formation of a white precipitate upon the addition of potassium mercury iodide. Isacoff (*28*) reported experimental results on the codistillation of propylene glycol with various hydrocarbons.

Stafford, Francel, and Shay (*64*) described a method for the identification of dicarboxylic acids in polymeric esters through the preparation of the dibenzylamides. This method, with modifications, should prove of value for the identification of the esters of dicarboxylic acids employed as adulterants for essential oils.

Collett (*9*) presented a procedure for detecting residual acetone in oleoresins based on a color reaction with salicylaldehyde.

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FOOD

JOHN R. MATCHETT

Bureau of Agricultural and Industrial Chemistry, Washington 25, D. C.

THIS review covers the period from about December 1949 to November 1950. It is a sequel to the review of methods of food analysis by Oser (*132*) for the period November 1948 to November 1949.

MOISTURE

Accurate and rapid methods for moisture in foods continue to attract the attention of analysts. The hope of finding one method applicable to all foods remains remote, although a technique using the Sicco-Rapid apparatus is said to be applicable to a large variety of foods (*21*).

The Karl Fischer reagent has proved to be of value in determination of moisture in such foods as cereals, starches, dehydrated vegetables, egg powder, and sirup mixes. A method employing azeotropic distillation and subsequent determination of water in the distillate by titration with the Karl Fischer reagent has been applied to oils containing as little as 0.002% water (*154*). A simplified method for standardization of the reagent has been proposed which avoids the preparation of absolutely anhydrous methanol (*153*).

Methods and equipment used for the determination of moisture have been the subject of a conference (*2*) and of a review paper (*58*). Reference methods suitable for calibration of empirical or indirect moisture methods for dehydrated foods were critically reviewed and arguments presented to show that the equilibrium vapor pressure of water may be a better index of stability of such foods than the moisture content (*110*).

A simplified method, developed for moisture in whole grains, involves heating in air for 16 to 18 hours at 130° C. (*143*). A mechanical device, consisting of a torsion balance combined with a drying element for measuring the moisture in flour, has been reported (*40*). Determinations can be completed in 5 minutes with an accuracy of about 0.1 to 0.2%. Results of moisture determination by the Association of Official Agricultural Chemists air- and vacuum-oven procedures on 10 samples of flour and flour mixes were compared by 14 analysts in five laboratories (*197*). Good agreement was obtained by analysts within a given laboratory, but considerable discrepancies were found among different laboratories. Deviations were less in samples run by the air-oven method than by the vacuum-oven method. A method for determination of moisture in potato starch has been reported (*184*) which involves giving the material a preliminary drying in a freely ventilated oven at 130° C., followed by desiccation beside phosphorus pentoxide in vacuo at 130° C. The determination requires about an hour.

Air- and vacuum-oven methods were employed to determine moisture in solutions of sucrose, glucose, and fructose of different acidities (*120*). Extent of decomposition and required drying times were established for various temperatures (from 60° to 98° C.) when drying with various additives such as pumice, sand, Celite, or paper pulp. A rapid and simple procedure, employing calcium carbide, has been developed for moisture in fresh or frozen sweet corn (*195*). Results agree with the vacuum-oven method within ±1%. Of particular value is the fact that the method can be used by the quality control group in processing plants.

Instruments are now fairly widely used for rapid moisture determination in certain foods. In general, such instruments utilize variations in the electrical properties of hygroscopic materials, and are useful only when the moisture content is not more than about 25%. A recent paper (*168*) appears to establish the feasibility of use of nuclear absorption phenomena as the basis of an instrument for rapid moisture determination over a moisture range varying from near dryness to saturation. The results for apple and potato tissues showed that energy absorption varied linearly with the amount of water in the tissues.

PROTEINS AND AMINO ACIDS

Methods of estimating total nitrogen of proteins by the Kjeldahl procedure continue to be investigated and the most important unsolved problems concerned with this method have been well summarized (*91*).

A procedure for the Kjeldahl determination of nitrogen in nicotinic acid and tryptophan has been investigated in collaboration with 16 independent laboratories (*198*). The method employed appears to be satisfactory and applicable to easily decomposed as well as refractory compounds. It has been observed that the rate of digestion of nicotinic acid was roughly doubled for each 10° C. rise in boiling temperature (*130*). Importance of the concentration of potassium sulfate in the digestion mixture has been stressed (*130, 166*).

Improved designs for digestion and distillation equipment have been reported (*46, 92, 171*). Minor improvements have been suggested for various macro, micro, and ultramicro-Kjeldahl procedures (*39, 101, 121, 129*).

A rapid method for estimating protein in cows' milk depends on determining ammonia liberated in alkaline solution, due largely to the glutamine and asparagine content of milk proteins (*96*). The biuret reaction has been used for the colorimetric estimation of proteins in fish (*173*).

Dilute alkali (0.1 to 0.2% sodium hydroxide) has been recommended for the extraction of protein from plants (*93*). The alkali has no apparent harmful effect on the constituent amino acids. Fish proteins have been isolated by dilute salt solutions (*49*). A better system has been developed for the electrophoretic separation of the known protein components in egg white, including the easily precipitated ovomucin (*55*). Simple, convenient, and inexpensive techniques have been elaborated for the electrophoretic separation of amino acids, peptides, and proteins on filter paper (*35, 47, 183*) and agar jelly (*4*). Location of the positions of the components is done by ninhydrin, Pauly reagent, or various dyes.

Chromatographic methods for the separation and estimation of proteins are still largely in the developmental stage. However, it appears likely that this field will receive greater emphasis during the next few years, because chromatographic techniques are rapid and convenient and do not necessarily require expensive equipment and highly trained technicians.

Three major components in egg white proteins have been separated on a cation exchange column (*174*), the resolved fractions being identified by refractometric optical measurements on the effluent immediately above the ion exchange column. Both one- and two-dimensional filter paper techniques have been employed for the chromatography of other proteins (*202*).

The status of chromatographic methods for the determination of amino acids has recently been reviewed (*155*). The precision of quantitative starch and filter paper chromatography procedures was estimated at 3 and 5 to 15%, respectively. Separation of amino acids on ion exchange columns has been studied (*28, 86, 104, 134, 135*), and chromatographic procedures have been elaborated for the separation of the dinitrophenyl derivatives of the amino acids on filter paper (*12, 122*) and columns of chlorinated rubber (*108*). Superior separations are claimed for the latter

technique. Most of the amino acids may be regenerated from their dinitrophenyl derivatives by treatment with 2 *N* sulfuric acid containing a few drops of hydrogen peroxide or by heating in a sealed tube with saturated barium hydroxide (*118*). Separation of all of the naturally occurring amino acids from each other on one-dimensional filter paper chromatograms using buffered, water-immiscible solvents and buffer-impregnated filter paper strips has been successful (*108*). This is particularly significant because it provides the basis for rapid and convenient quantitative estimations of amino acids in protein hydrolyzates. A proportional divider (Partogrid) for rapid determination of R_f values on one-dimensional filter paper chromatograms (partograms) has been suggested (*156*). Other methods have been reported recently for the quantitative determination of amino acids by filter paper chromatography (*15*, *177*). While their applications to foods have been limited, it is likely that greater use of these techniques will be made once reliability has been evaluated.

The principal applications of filter paper chromatography to food analysis have been of a qualitative nature. A new amino acid, γ-aminobutyric acid, has been found in extracts of beet root (*187*), yeast (*146*), and apple pulp (*82*). β-Alanine has been reported in extracts of apple pulp (*82*). Glutathione has been identified in an alcoholic extract of peanut kernels (*147*). Numerous free amino acids have been reported in citrus juices (*157*), and extracts of apple, pear, prune, apricot, and avocado (*82*). Rockland and Dunn's rapid test tube chromatography technique has been modified for the estimation of monosodium glutamate and vegetable protein hydrolyzate in soups (*137*).

Chemical methods for amino acids have recently been reviewed (*131*).

Studies on the determination of amino acids by an ingenious isotope derivative technique have been continued (*89*), and the radioisotope carrier technique has been employed for the determination of alanine, glycine, and proline in α-, β-, and γ-caseins (*65*). Whole casein was shown to contain less than 0.1% hydroxyproline. The isotope techniques are not likely to be used for routine determinations of amino acids in proteins and foods because of the great number of manipulations and special equipment required. Peri-naphthindan-2,3,4-trione hydrate has been used in a titrimetric ammonia method for the estimation of amino acids (*124*). A reinvestigation of Pope and Stevens' method for determining amino acids by means of iodometric titration of their soluble copper salts has led to an improved method using washed copper phosphate (*164*). By its use 14 of 19 naturally occurring amino acids were determined with a comparatively high degree of accuracy.

Aspartic acid in protein hydrolyzates has been determined by conversion of the aspartic acid to fumaric acid with dimethyl sulfate, followed by colorimetric estimation of fumaric acid (*57*). Cysteine and cystine have been determined by argentometric amperometric titration (*97*). A colorimetric method employing *p*-dimethylaminobenzaldehyde has been reported for the estimation of hydroxyproline (*128*). The procedure requires only about 1 to 2% as much protein as has been necessary with previous methods.

Methionine has been determined in a number of grains and legumes by a modified McCarthy and Sullivan sodium nitroprusside procedure (*159*). *p*-Dimethylaminobenzaldehyde has been used for the colorimetric estimation of tryptophan in unhydrolyzed proteins (*175*). This reagent has been used in the past for determination of tryptophan in alkali-digested proteins. Values obtained for the tryptophan content of unhydrolyzed casein and egg albumin were considerably higher than those found in the alkaline hydrolyzates. Glutathione has been determined polarographically in citrus juices and in extracts of potato and guava (*33*).

Results have been published of a collaborative study by 12 independent laboratories, on the microbiological assay of 16 amino acids in a synthetic test mixture and in hydrolyzates of whole egg, egg albumin, casein, wheat gluten, and peanut flour (*160*). The absolute mean deviation reported by nine laboratories for the analysis of the synthetic mixture varied between 6.7 and 11.3%, while the absolute mean deviation for individual amino acids varied between 0.4% (glutamic acid) and 5.6% (arginine).

It has been observed (*151*) that *Leuconostoc mesenteroides* P-60 and *L. citrovorum* 8081 are capable of utilizing peptides released by in vitro enzymatic hydrolysis of proteins. The use of these organisms in determining amino acids in unhydrolyzed or partially hydrolyzed biological materials does not seem advisable.

Improved procedures have been elaborated for the microbiological assay of cystine with *L. mesenteroides* P-60 (*25*) and tryptophan with *Streptococcus faecalis* (*117*). A procedure for the microbiological assay of L-, D-, and DL-methionine has been suggested (*24*). DL-Methionine can be assayed with either *L. arabinosus* or *Lactobacillus fermenti*, while L-methionine can be determined with *L. mesenteroides* P-60. D-Methionine may be estimated by difference.

METALLIC IONS

Because iron is an important constitutent of foods and is included in some form in the enrichment of cereals, flour, and bread, a rapid and accurate method for its determination is of interest. Values that check well with the A.O.A.C. method have been obtained by a technique involving the muffle ignition of the sample with magnesium nitrate (*178*). This ashing method causes no loss of iron, and possible interference by pyrophosphate is overcome by the presence of considerable quantities of magnesium, and by acid hydrolysis. Iodine determinations of foodstuffs are of value in any study of simple goiter. A quick colorimetric method involving the use of sodium nitrate and sulfuric acid has been suggested (*176*).

Quantitative values for sodium, potassium, and calcium in human and cows' milk have been obtained by using flame spectroscopy techniques (*90*). The method is said to be accurate to within 5%. Apple slices may be treated in a calcium chloride bath prior to canning, for the purpose of increasing their firmness. A rapid control method for maintaining the concentration of calcium chloride in the bath has been devised by modifying the soap method for measuring the hardness of water (*193*).

Sodium chloride determinations in meat extracts and yeast hydrolyzates are important control measures. A rapid technique, requiring about 30 minutes, consists of deproteinizing the food with Carrez reagent and determining chloride in the protein-free filtrate with Volhard reagent (*61*).

In the determination of magnesium by the use of thiazole yellow, errors due to lack of consistency in the degree of dispersion of the red colored coordination complex seem to have been circumvented by a new approach to the problem (*83*). An accurately measured quantity of thiazole yellow was added to the magnesium solution and excess dye removed from the reaction mixture with *n*-butyl alcohol. A calibration curve is prepared relating quantities of magnesium directly with color intensity of the *n*-butyl alcohol solution of the excess dye. The method is applicable to milk, plant tissue, soil extracts, and blood serum.

Colorimetric methods have been described for the determination of copper in the ash of food products (*64*, *69*). One procedure (*69*) uses 1,8-dehydroxy-3-methylol anthraquinone, and in the other (*64*) pyridine and salicylic acid are utilized.

FATS

All methods for measuring the iodine number of oils require long absorption times varying from 30 minutes to 2 hours. For rapid control work of continuous plant operation, this is too long. A recently published modification of the Rosenmund-Kuhnhenn method should be of interest to those concerned with plant operation (*5*). Iodine numbers can be obtained in 1 minute with all ordinary nonconjugated fats and oils. The method seems to

agree well with the Hanus method except in the case of tung oil. Agreement was also obtained with the Wijs method in the case of oils of low iodine number, but values were slightly higher in the case of oils having iodine numbers of more than 100.

A centrifugal method for determining fat in cacao products has been suggested (*94*).

A rather extensive review with 74 references dealing with cholesterol determination will be of interest to those working in this field (*59*).

ENZYMES

The role of phospholipides as they affect the consistency of foods, and their susceptibility to probable taste changes brought about by oxidation and rancidity, is well known. A method of detecting enzymatic breakdown of phospholipides, based on the fact that serine and ethanolamine alone of the basic components of phospholipides liberate ammonia on oxidation with periodate, has been reported (*158*). One method of expressing the activity of papain is by the Balls-Hoover milk clotting units. A more recent method is based upon the liberation of tyrosine from a standard solution of ovalbumin (*51*). The papain unit is defined as that amount of enzyme which in 10 minutes at 36° C. liberates all the tyrosine from 1 mg. of dry egg albumin. A photometric ninhydrin method for measurement of proteolysis has also been reported (*165*).

One of the first phosphatase tests for distinguishing pasteurized from raw milk was presented in 1934. Since then the test has been improved, and in 1947 it became known as the Sanders and Sager phosphatase test. More recently (*116*) a new technique has been developed for this test. The method depends on the formation of red phenolphthalein color from enzymic cleavage of phenolphthalein diphosphate (sodium salt) at pH 9 to 10 in the presence of a little chloroform with incubation at 37° to 38° C. If no red color is visible after 24 hours, phosphatase is absent.

CARBOHYDRATES

In the determination of reducing sugars in plant materials, the alcohol-extracted sugars in solution are usually clarified with neutral lead acetate to remove nonsugar reducing substances, and excess lead is removed with disodium phosphate. A study (*10*) of the alcoholic extract of some 29 fruits and vegetables showed that in almost half of them determination of reducing sugars, by the A.O.A.C. copper micromethod and the Hassid ferricyanide micromethod, could be carried out without removal of alcohol and without any form of clarification. The other half required treatment of the alcoholic extract with a selected decolorizing carbon only.

A modified Coalstad electrometric titration method for determining the end point in the Lane and Eynon procedure has been reported (*6*). Platinum was substituted for copper in one of the electrodes. A carbonate-buffered cupritartrate reagent permits the determination of reducing sugars to an accuracy of a few tenths of 1% (*72*). The method is applicable to the determination of reducing sugars in refined sucrose.

A method useful for the measurement of lactose in bread (*27*) and a picric acid procedure applicable for both lactose and sucrose in dairy products were reported (*140*). The Bertrand method has been modified for determination of 0.8 to 40 mg. of sugar per sample of plant extracts (*103*). The usual time-consuming chemical methods for determining levulose have been superseded by a polarographic technique (*194*), and the polarograph has also been used to measure the surface-active impurities in sugar (*185*).

A complete report of handling, sampling, classification, and analysis of raw sugar was presented before the International Commission for Uniform Methods of Sugar Analysis in 1949 (*84*).

An optimum procedure for preparing sucrose liquor and sirup for color measurements has been proposed (*115*). Equations and a nomograph relating the reducing power and values for double polarization of sugar solutions have facilitated the approximate determination of mixtures of any two known reducing sugars and sucrose (*152*). The method is useful for analysis of chocolate, sugar sirups, and the like.

A general qualitative test for carbohydrates (*41*) and several qualitative tests for fructose or other ketohexoses have been presented (*53*, *66*, *114*, *203*). Disches' carbazole method was modified to permit differentiation between galacturonic and glucuronic acids (*44*). Sugars did not interfere in the test. A qualitative scheme for the identification of sucrose, glucose, fructose, maltose, and lactose in macro amounts, based upon solubility differences and specific color reactions, was presented (*189*). This method may be useful for occasional qualitative identifications.

Triphenyltetrazolium chloride dissolves in water to form a colorless solution. After being reduced, this salt forms triphenylformazan, which is a red, water-insoluble compound. Reduced triphenyltetrazolium chloride has been used for the quantitative colorimetric determination of lactose in milk and for glucose and fructose in honey (*113*). Microdetermination of sugars applicable to the study of spots on paper chromatograms involves preparing paper strips in duplicate. Location of sugar spots on one is determined by the Partridge or Horrocks method. Corresponding parts of the other strip are cut out and the sugar is extracted from each individually with methanol, the methanol is removed by evaporation, and the sugar in the residue is determined colorimetrically by a modification of Partridge's aniline phthalate method (*14*). The method is suitable for pentoses and aldohexoses but not ketoses.

Recent reviews of paper chromatography of the carbohydrates (*34*, *196*) emphasize the possibilities of extending this technique to the food field. It need no longer be necessary for food chemists to express their reducing values as glucose without having definite knowledge of the identity of the sugars in their material. Techniques have been presented that may allow a tenfold extension of the quantities now separable by paper chromatography (*125*, *203*). The properties of 22 filter papers were tested for use in chromatography (*98*). At present most of the known sugars and many of their derivatives have been separated by paper chromatography, eluted from the paper, and analyzed. An improvement in elution technique allows simple, rapid, and quantitative removal of the separated sugars from the developed chromatogram (*102*). It has recently been shown that the best separation of sugars occurs when paper chromatograms are run at 37° C. (*80*). Several interesting experiments are offered, one of which is the separation of α- and β-methyl rhamnosides. Other workers have presented improvements in procedures developed for their particular needs (*8*, *16*). For instance, paper chromatography has been employed to separate the radioactive products formed during photosynthesis in $C^{14}O_2$, and for the separation and identification of carboxylic acid and phosphate esters (*8*). A multiple development in one direction permitted the resolution of a homologous series of oligosaccharides up to a degree of polymerization of about 10 glucose units (*85*).

Qualitative paper chromatography has been used to identify glucose, fructose, and sucrose in lemon, orange, and grapefruit juices (*107*). The presence of sucrose in lemon juice (about 0.05%) was demonstrated but was undetected by chemical analysis. Sugar beet juice has been analyzed quantitatively for raffinose by paper chromatography (*1*, *42*). This would have been an enormous task by any other method. The use of paper chromatography for the separation and quantitative analysis of the sugar-phosphate esters has been reported (*31*).

The chromatography of the sugars on charcoal using dilute alcohol was reported (*188*), and this work should be of interest to anyone using charcoal to remove "nonsugar" reducing substances.

A procedure was reported (*106*) in which starch was extracted from sugar-free vegetable material with cold dilute perchloric acid, and both starch and amylose were determined colorimetrically. Anthrone was used in a like manner for the quantitative analysis of animal glycogen (*167*). These procedures all offer the

advantage that no previous acid hydrolysis of these polysaccharides is necessary, because hydrolysis and color development in the strong sulfuric acid occur at the same time. A note on the specificity of the anthrone reaction (*161*) reviews the requirements for the anthrone–sugar–sulfuric acid reaction.

A method for the approximate determination of pectin in raw beet sugar was presented (*192*). The method calls for saponification of the pectin, distillation of the methyl alcohol, oxidation of the alcohol with chromic acid, and back-titration with thiosulfate. A method of measuring the jellying power of pectin is based on the sagging of a piece of jelly under its own weight (*45*). Measurements of gel strengths by using the Luers-Lochmuller "pectinometer" (*68, 74*) have been reported.

VITAMINS

The spectrophotometric method for vitamin A now appears in the U. S. Pharmacopeia (*142*), and calls for absorbancies of the unsaponifiable extract at wave lengths 310 mμ, 325 mμ, and 334 mμ. It requires that the ratio of the colorimetric (antimony trichloride) to the spectrophotometric assay values shall fall within the specified limits of 1.0 to 1.3 (*190*). A method for the determination of vitamin A in the presence of interfering substances has been reported (*109*) involving the use of 315 and 338.5 mμ as reference points rather than 310 and 340 mμ for correcting for extraneous matter absorption by the Morton and Stubbs method.

Study of a better method for vitamin A in feeds was continued, and a technique in which 30% acetone in hexane was used for extraction of vitamin A from standard samples followed by chromatography on magnesium oxide and subsequent determinations with antimony trichloride gave results which correlated fairly well with the potencies of the standards (*162*).

A biological method of vitamin A assay which perhaps is more of fundamental interest than of practical application, at least at present, is based on the degeneration of nerve tissue of the central nervous system of rats in vitamin A deficiency (*30*). Such lesions are present to varying degrees in rats that are still growing and from external appearance are in good condition. Abnormalities, detected by histological examinations taken as the basis of evaluation of the prophylactic assay, which covers an 8-week period, indicated that the procedure was at least as accurate as the 3-week curative growth test.

A collaborative study by the Association of Official Agricultural Chemists on the determination of carotene in alfalfa has been continued (*144*). One hundred and ten laboratories have participated in the study of the chromatographic procedure with a final recommendation that overnight extraction at room temperature be used as an alternative to refluxing for 1 hour, that a more uniform adsorbent be sought, and a procedure for purification of solvents be devised. The use of a mixture of equal parts of toluene, ethyl alcohol, and ethyl acetate for giving a complete extraction of carotenoids from feeds was suggested (*32*). The chromatographic separation of carotene from other pigments was similar to that of the Association of Official Agricultural Chemists. This procedure avoids saponification, as does another for carrots, spinach, and red pepper (*60*) where carotenoids were extracted and chromatographed directly. A method for estimating carotene and anatto in the presence of dimethylaminoazobenzene by means of paper chromatography has been suggested (*43*).

A rapid two-stage chromatographic method applicable to the determination of carotene in dried peas has been reported (*56*).

Considerable research has been undertaken on assay methods for vitamin B_{12}, and in the United States a recent collaborative study sponsored by the U. S. Pharmacopeia Anti-Anemia Board has been the basis for adoption of an official procedure. Reports on microbiological techniques seem to have been limited to *Lactobacillus leichmannii* ATCC 4797 (*11, 138, 163, 172, 180*) and strain 313 (*172*). Media containing crystalline amino acids as a nitrogen source and adsorbed tomato juice filtrate (*138*) and fumaric acid (*181*) as growth stimulants have been studied. Microbiological assay methods applied to alfalfa will give misleading results, because there are present factors other than vitamin B_{12} to which *L. leichmannii* respond (*11*). It appears likely that these factors may consist of the naturally occurring desoxyribosides, which have been shown to respond to the B_{12} assay.

Chromatographic methods have been proposed for the separation of compounds possessing vitamin B_{12} activity (*95, 201*). Butyl alcohol was suitable only for the separation of B_{12} from either adenine-desoxyribose nucleoside or thymidine. Phenol was not suitable for separation (*95*). Conditions have been defined for separating B_{12} and B_{12a} from mixtures of these two substances (*201*). The reduced forms of B_{12}, called B_{12a} and B_{12b}, are known to be identical.

Rat (*148*) and chick (*29*) assay methods for B_{12} have been reported. Techniques for preparing B_{12}-depleted chicks for use are described (*13*). A colorimetric method is based on the color of a hydrolytic product of B_{12} after preferential extraction to separate it from impurities (*52*). Results are in agreement with those obtained by microbiological methods.

Published data on recent improvements in the estimation of vitamin E are lacking except for one report based on the spectrophotometric determination of α-tocopherol in the presence of γ-tocopherol (*139*). The problem of determining tocopherols is complicated by the plurality of tocopherols having different activities, by the interference of other inactive compounds, and by the instability of tocopherols during manipulation.

Pantothenol and pantothenates assume importance as members of the vitamin B complex, and a rapid colorimetric method has been described for estimating pantothenates in tablets and ampoules (*200*). Application of the method to food products is not mentioned by the authors. Efforts have been made to use differential assays of free and total pantothenic acid with *L. arabinosus* before and after suitable enzyme treatment as a guide in attempts to concentrate the bound forms from yeast and liver (*127*).

A number of colorimetric techniques for choline involving the use of Reinecke's salt have been suggested. Recent developments include a gravimetric method utilizing the same reagent (*133*). When this method is used in assaying folic acid–containing liver fractions, falsely high results are obtained (*3*). This is due to the fact that such fractions contain a basic substance which precipitates with ammonium reineckate. The "liver" reineckate is, however, insoluble in acetone, while choline reineckate is soluble, thus offering a means of separation (*3*).

A spectrophotometric method for vitamin C has been described (*111*) and methods for vitamin C in the presence of ferrous salts have been investigated (*20, 81*), particularly from the standpoint of accurate vitamin C determinations in canned foods. Means of stabilizing the standard solution of 2,6-dichlorophenolindophenol are suggested (*81*) and the use of a sodium acetate–hydrochloric acid buffer solution or of a 10% acetic acid–0.1% oxalic acid solution as an extracting medium obviates difficulties due to the presence of iron or copper (*20*).

A new book on vitamin analytical techniques, presenting the physical, chemical, microbiological, and animal assay methods employed, should be of interest to those working in this field (*70*).

COLOR, ODOR, AND TASTE

A method giving approximate color values of tomato paste involves a color index obtained by measuring the color of extracts of the paste with a photoelectric colorimeter instead of by reflectance measurements (*38*). The method may have broad application to many kinds of foods such as citrus products, avocados, dates, and peppers. Another method consists of determining the color with a Hunter color-difference meter and then interpreting specifications on a special chromaticity diagram (*204*). Because the procedures are objective and unrelated to the ability of the operator to match or evaluate colors, the personal factor is eliminated.

Degree of nonenzymatic browning of some dehydrated vegetables during storage has been measured (*73*) as the difference between optical density values of extracted soluble color materials from samples before and after storage.

Reflectance spectrophotometer and photoelectric reflectometer measurements have been used to study and determine the extent to which original natural color is preserved during processing and subsequent storage of certain foods (*50, 112*).

A photoelectric method for assessing flour color as an indicator of flour grade comes from England (*88*). An apparatus has been developed for this, the readings of which are independent of the effect of natural or artificial bleaching.

Permanent glass color standards have been developed for use in a simple color comparator for grading the color of maple sirup (*19*).

The chemistry of flavor has been the subject of a recent review (*145*), and flavor profiles have been discussed in another recent publication (*23*). A rather comprehensive study of odors is also of interest in this connection (*179*). One of several suggestions for improvement in flavor evaluation procedure has been a scoring system devised to differentiate between intensity of abnormal or undesirable attributes and preferred intensity of typical or desirable attributes of food (*79*). The system has been found to provide reproducible results related to composition of the test materials. Another procedure based on paired and triangle difference tests emphasizes the use of "warm-up" samples, reference standards, rinses, and control of the quantity judged and of the time interval between judging of samples (*141*).

Among investigations of methods applicable to specific foods is the development of a procedure for evaluating oils (*123*), and the objective and organoleptic evaluation of quality in raw and canned peas (*99*).

A study undertaken to determine the reason for erratic results in judging sulfited foods showed that tasting one sample of mashed potatoes containing 12 to 100 p.p.m. of sulfur dioxide dulled the acuity of the judges for detecting subsequent sulfited samples (*17*). The authors recommend evaluating only one sulfited sample per judging session until a more reliable technique is developed.

Some of the difficulties involved in judging flavor of foods that can be identified by color differences are illustrated in a report of an evaluation of spices, spice oils, and oleoresins (*126*).

SPOILAGE AND CONTAMINATION

Methods of determining insect counts in foods have been the subject of three recent papers (*62, 170, 181*), involving an improvement over the older Wildman method, extraction of the product with castor oil followed by gasoline, or preferential staining which leaves unchanged the insect fragments so that they appear in their natural color.

Methods of the National Cheese Institute and the Association of Official Agricultural Chemists for determining extraneous matter in cheese have been modified for use with Swiss products (*87*).

The conventional Schechter-Haller colorimetric method for DDT is not applicable in the presence of more than traces of fat. It has been found (*37*) that Celite impregnated with sulfuric acid–fuming sulfuric acid, and slurried with carbon tetrachloride will hold fats within a chromatographic column, while DDT will pass through with the carbon tetrachloride. From 90 to 100% of DDT added to butter oil was recovered with this method.

A colorimetric method for estimating trimethylamine is of interest in fish decomposition (*48*), and the presence of succinic acid has been shown to be an index of decomposition in tuna (*77*).

Extensive decomposition experiments have been carried out on cream and data presented on butyric and water-insoluble acids (WIA) in cream and butter (*75*). Individual volatile acids as evidence of decomposition have been applied to canned tuna (*76*), the results indicating that these acids are a good index of the stage of decomposition of the raw material from which the canned product was prepared.

Shaking samples of potato flakes, flour, and starch flour with chloroform in order to determine sand has been found to be undependable (*186*). A better procedure makes use of hydrolysis of the starch with hydrochloric acid, and this is even preferable to ashing (*186*).

Methods of detecting Cysticercus-contaminated meat have been suggested (*100*). Cysticerci representing the larval forms of *Taenia solium* (pork tapeworm of man) and *Taeniarhynchus saginatus* (tapeworm of man), either dead or alive, give a pink fluorescence on illumination with ultraviolet of specific wave length. The property of luminescence has also been applied for detection of impurities in other foods such as fruit juices, vinegar, vegetable oils, butter, and flours (*182*).

The possible presence of coliform organisms in citrus juices has become of increasing importance during the past year, and apparently satisfactory methods remain still to be worked out as applied to these commodities. It has been reported (*199*) that various organisms meeting the definition for coliforms persisted in frozen concentrated orange juice for 43 weeks. Most were Aerobacter species, but some resembling *Escherichia coli* were found. In a survey made of citrus processing plants in Florida during the 1948–49 season, no *E. coli* was found in any of the samples studied (*136*). This paper points out the importance of the media used.

MISCELLANEOUS

Methods have been suggested for measuring the firmness of red cherries (*191*), calcium-treated apples (*67*), and corn (*7*).

The detection of egg in salad cream is based on a colorimetric method of determining choline derived from egg lecithin by acid hydrolysis and conversion of the choline into its pink reineckate (*36*). The old Strohecker method has been modified for the determination of rye flour in mixtures with wheat flour (*63*), and a laboratory procedure for evaluating curd-producing capacity of soybean products has been described (*105*). Curd volume is an accurate index to the per cent of soluble protein in soy flours.

A spectrometric method for estimating solids in tomato products has been proposed (*177*), the results having additional value in that they can be used as a criterion of the degree of ripeness of the tomatoes used.

A rapid method for determination of crude fiber has been reported (*71*). Materials of high fat content must first be defatted to avoid error that may be caused by foaming.

Propylene glycol is used in many food preparations and particularly in imitation vanilla extracts. The usual separation of propylene glycol by direct distillation has been found unsatisfactory because of poor recovery and charring of sugar when present. Extraction methods have been found equally unsatisfactory. A procedure depending upon the isolation of propylene glycol from vanilla extract by codistillation with an organic solvent such as heptane has been reported (*22*). The method can be used when propylene glycol occurs alone or in the presence of glycerol.

The A.O.A.C. Power-Chestnut and Baily-Andrew methods for caffeine in coffee and tea are time-consuming. A semimicromethod has been suggested requiring only 15 to 25% of the time necessary for the A.O.A.C. method (*18*). An improved method for determining theobromine in cocoa products is also of interest (*78*).

Congressional action in repealing the tax on oleomargarine made it evident that some method would have to be found to differentiate between butter and margarine. A test has been suggested (*54*) to differentiate butter from margarine, based on the "critical temperature of dissolution" (CTD test) first enunciated by Valenta over 55 years ago. In the proposed test a mixture of ethyl and isoamyl alcohols of specified strength is used as a solvent of the oil from the butter or margarine. Upon heating and stirring the mixture becomes clear and homogeneous. The mix-

ture is then cooled while being stirred and the temperature is noted at the first discernible turbidity. The magnitude of temperature spread at which butter and margarine become turbid was significant enough, even in the hands of different analysts, to distinguish between the two commodities.

The toxic factor derived from "agenized" (nitrogen trichloride-treated) flour has been investigated by several laboratories (*9, 26, 119, 149, 150, 169*). The sulfoximine of methionine has been tentatively identified as the toxic factor (*119, 169*). Use of nitrogen trichloride for flour bleaching is no longer permitted in the United States.

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FERROUS METALLURGY

H. F. BEEGHLY
Jones and Laughlin Steel Corp., Pittsburgh, Pa.

PRACTICAL advances in ferrous analytical chemistry stem from the need for more reliable and specific data on raw materials and their composition at different stages of refining into finished products. Each new raw material utilized and each new alloy developed presents an analytical problem that must be solved successfully if its commercial value is to be realized.

GENERAL INFORMATION

The pH conditions necessary for complete extraction of such metals as aluminum, copper, ferric iron, manganese, molybdenum, nickel, and stannic tin from aqueous solution with a 1% solution of 8-hydroxyquinoline in chloroform were investigated by Gentry and Sherrington (*29*). Their results were different from those of Moeller (*55*). Applications are suggested for extraction of heavy-metal impurities from analytical reagents to be used in trace analyses. Because the extraction conditions used are nonselective, the chloroform–8-hydroxyquinolate extraction has no special advantage for direct quantitative analysis except in the case of stannic tin. In this case traces of tin can be separated by distillation and concentrated in the yellow chloroform layer. Chernikov and Dobkina (*15*) made a similar examination using diethyldithiocarbamate with ethyl acetate as extractant. Nachtrieb and Fryxell (*60*) studied the extraction of gallium from aqueous solution. Gorkiĭ (*32*) evaluated several nonaqueous solvents for analytical work.

Lester (*47*) and McDonnell and Wilson (*49*) reviewed use of organic reagents for microanalysis and compiled a list of metalo-organic compounds suitable for final weighing in microgravimetric analyses. In a series of articles, Nutten (*63*) considered the various substances that have been advanced for use as primary standards. Stock and others (*77–79*) in a series of articles gave an account of miscellaneous microchemical devices useful for handling the unusual analytical problem. Stock also described (*76*) simple conductometric titration apparatus using platinum wire microelectrodes with which volumes of 1 ml. or less may be titrated. Stross (*81*) depicted a number of handy devices for the analytical laboratory that handles large numbers of samples regularly. Cunningham (*18*) outlined micromethods and techniques primarily for use in nuclear research, but some of them have general applicability in instances where small quantities of material must be evaluated.

Beck (*3*) devised a gravimetric procedure in which the precipitate is separated by centrifuging. A crucible is attached to the bottom of the centrifuge tube by a ground joint. Then the crucible containing the precipitate is removed, dried, and ignited. This apparatus may be useful for the determination of amorphous, gelatinous, or very finely divided precipitates. Croall (*17*) commented on applications of perchloric acid in the analysis of steel works materials.

Flagg, Liebhafsky, and Winslow (*22*), Furman and Garner (*26*), and Moeller and Cohen (*56*), respectively, presented new data on the absorption spectra of zirconium lakes, trivalent and tetravalent vanadium ions under various conditions, and the 8-quinolyl chelates of the Group III B elements.

Maxwell and Graham (*51*) published a useful review with 181 references on the mercury cathode and its applications; newer techniques in analytical chemistry were surveyed by Nicholls (*62*). Furman (*25*) reviewed electrical methods of chemical analysis.

Birks and Brooks (*7*) applied the x-ray fluorescence method to the quantitative determination of hafnium in zirconium and of tantalum in columbium without separation. Quantities in the order of 0.1% were said to be detectable with a quantitative accuracy on the order of 4% of the amount present. A number of new techniques for measuring the thickness of metallic protective coatings on steel have been devised (*4, 24, 82*).

Hofer, Peebles, and Guest (*38*) described apparatus and techniques useful for preparation of powder specimens for x-ray diffraction analyses. Jaycox (*39*) developed techniques utilizing spectrochemical buffers. He explained methods of sample preparation and defined excitation conditions for analyzing virtually all types of inorganic materials. Woodruff (*86*) utilized briquetted powders, drillings, etc., as specimens for the routine spectroscopic analysis of steels; such elements as tin, lead, manganese, nickel, chromium, molybdenum, cobalt, titanium, aluminum, zinc, and boron may be determined. Operational details and precision and accuracy over a 5-year period are outlined.

A number of books of value to the ferrous analytical chemist were published. These for the most part deal with the physical aspects of analysis, such as spectroscopy and instrumentation (*6, 8, 14, 48, 53, 54, 59*). The need for a new, comprehensive book on ferrous metallurgical analysis apparently remains unfilled.

A valuable addition to the analytical literature is the revised and supplemented x-ray diffraction data cards and index (*1*) published by the American Society for Testing Materials.

METHODS FOR THE ELEMENTS

Aluminum. Aluminum was determined polarographically by Jessop (*40*), who utilized the catalytic reduction in the presence of excess bromate to obtain steps at −0.35 and −0.78 volt against a saturated calomel electrode in 0.1 molar potassium chloride. The method is said to be suitable for determining aluminum in the range of from 0.01 to 0.5 mg. per milliliter. Raine (*69*) utilized 8-hydroxyquinoline in benzene to determine aluminum colorimetrically. He extracted at pH 2.0 to 2.5 the iron remaining after a preliminary separation and then extracted the aluminum complex at pH 5.0. Shemyakin and Barskaya (*72*) separated interfering elements with the mercury cathode and reacted the aluminum with diamino bright blue FFG in a weakly acidic solution to provide the basis for a colorimetric method. Conditions for development of color are not given.

Short (*75*) outlined a method intended for determining quantities of aluminum as small as 0.001% in high-purity iron with an accuracy of ±0.0005%. The method involves separation of iron and other interfering elements with ether, followed by a chloroform extraction of cupferron complexes at pH 0.5. Aluminum is then determined in the remaining aqueous layer by means of Aluminon under carefully controlled conditions.

Beryllium. Beryllium was determined in the presence of large amounts of iron, aluminum, and manganese by Leibowitz and Young (*46*).

Boron. Boron in magnesite and fused magnesia may be determined by a method devised by Hazel and Ogilvie (*37*), entailing solution of the sample in hydrochloric acid under a reflux condenser, followed by precipitation of impurities with sodium carbonate and sodium hydroxide. Boron is titrated in the purified solution with 0.1 *N* sodium hydroxide in the presence of mannitol. The method is said to be free from interference from the usual impurities in magnesite.

Calcium. Calcium in small percentages was obtained spec-

troscopically in steel by Komaroviskiĭ (*44*). Mosher, Bird, and Boyle (*57*) utilized a flame photometer for determining this element in magnesite and brucite. They used the hydrochloric acid solution of the sample. Filtration, precipitation, and titrations were not necessary. The effect of the common impurities on results was investigated.

Carbon. Carbon in steel was determined by measuring the change in conductivity of a barium hydroxide solution in which the carbon dioxide from combustion of the steel was absorbed in apparatus described by Bennett, Harley, and Fowler (*5*). Full details of the procedure (intended for low-carbon steels) and novel features of the absorber and conductivity measuring bridge circuit are given. Résumés of methods in current use in different laboratories for determining carbon contents of less than 0.10% in ferrous materials also were published during the year (*23*, *28*). Difficulties that arise with the methods and the necessary precautions for their use are indicated.

Mazumdur and Ghosh (*52*) determined carbon in steel spectroscopically; values obtained were said to be within ±0.02% of the true values. They used a large quartz spectrograph with a voltage of 15,000, capacitance of 0.005 microfarad, inductance nil, and a spark gap of 2 mm. A pointed copper rod was used as an upper electrode; the lower steel electrode was conical with a flat top. For materials containing more than 0.40% carbon, dry nitrogen was blown through the spark gap.

Chromium. Furness, Hardwick, and Bryant (*12*, *27*, *36*) reported the results of investigations made as an outgrowth of efforts to coordinate methods for determining chrome in chrome ores in use by various government departments and metallurgical firms in England in a series of three papers. The first covers work on the effects of vanadium and arsenic on the volumetric determination of chromate. The second gives results for chromium on a synthetic sample of known composition using different methods. The third compares results obtained on a National Bureau of Standards chrome refractory with selected methods. A description of the procedure for each method is given. The British Iron and Steel Research Association (*9*) recommended as a standard procedure a method based on ammonium persulfate oxidation of chromium with a silver nitrate catalyst, followed by titration with standard ferrous ammonium sulfate and potassium dichromate with barium diphenylamine sulfonate as indicator.

Parks and Agazzi (*64*) titrated chromium and vanadium amperometrically at the rotating platinum electrode with a ferrous solution after oxidizing these elements by heating them with perchloric acid and permanganate. The method was said to have been applied successfully to steel. Urone, Druschel, and Anders (*83*) described a micromethod for obtaining the chromium content of dust with the polarograph. The lower limit of sensitivity is 0.2 microgram of chromium per milliliter of final solution. Bureau of Standards steel samples weighing from 1 to 10 mg. as well as dusts and chrome refractories were analyzed successfully.

Cobalt. Use of 3-nitrososalicylic acid to form at a pH of 5.6 to 6.0 a brown colored, petroleum ether–soluble cobalt complex and a red-colored, water-soluble nickel complex provides a basis for the separation and determination of these elements by a procedure described by Perry and Serfass (*66*). Cupric copper and ferrous iron interfere. At pH 4.0 copper forms a red complex with the reagent and can then be estimated also. The method was found satisfactory on steel alloys containing 5% or more of nickel and cobalt and for copper contents of 1% or more. Further work on steels and industrial applications of the reagent are to be described by the authors in a future publication. Shome (*73*) utilized the reaction of isonitrosomethylhydroresorcinol with cobalt as a basis for a colorimetric procedure for its determination.

Yardley (*87*) surveyed methods available for the accurate determination of cobalt with the object of securing a method suited to the routine assay of highly pure cobaltous salts. Special attention was paid to their potentiometric titration with potassium ferricyanide, which was found to give accurate results under the conditions described.

Copper. The chloroform-soluble blue precipitate formed when an aqueous solution of pyridine containing a large amount of salicylic acid is added to a solution of a copper salt may be made the basis of a method for quantitative analysis for copper, according to a procedure described by Gordieyeff (*31*). The reaction of copper with 3-nitrososalicylic acid has also been utilized for the determination of copper (*66*). The serious interference caused by iron, nickel, and cobalt is said to be eliminated by addition of a very large excess of ammonium bifluoride.

Iron. A rapid method described by Pepi (*65*) for determining iron in such high temperature alloys as Inconel and Vitallium is based on reaction of ferrous iron with 1,10-phenanthroline following solution of the sample in aqua regia containing a few drops of hydrofluoric acid. Hydroxylamine hydrochloride is used to reduce the iron. The reproducibility was found to be good and the average error to be ±0.02% of the iron present.

Diphenylamine, diphenylbenzidine barium diphenylamine sulfonate, ferrous phenanthroline, and potassium ferrocyanide were compared by Stockdale (*80*) as indicators for the estimation of ferrous iron by dichromate. Barium diphenylamine sulfonate was considered to give the most distinct end point and to be least affected by conditions of the titration.

Gases and Nonmetallic Compounds. Carney, Chipman, and Grant (*13*) described procedures and apparatus for sampling and analyzing steel for hydrogen. This method involves fusion of the sample in vacuum in the presence of tin and collecting the evolved gases. Guldner and Beach (*34*) developed improved apparatus for use with the vacuum fusion methods. McGeary, Stanley, and Yensen (*50*) utilized a rapid vacuum fusion procedure for determining oxygen in steel. Short (*74*) suggested determining oxygen in chromium metal by annealing it at 800° for 2 hours, cooling, and dissolving the matrix away with 10% hydrochloric acid to leave only the oxide. Low results are said to be obtained if the sample is not annealed or if stronger acid is used.

Vogel (*85*) reviewed methods for the determination of metallic iron and ferrous and ferric oxides in mixtures. A solution of 2.5% by volume of bromine in ethyl alcohol was considered best for determining elemental iron in the presence of its oxides. Neumann and Meyer (*61*) utilized a solution of 0.1 *N* sodium thiosulfate and ferric chloride for the isolation of inclusions from iron.

Popova and Rybina (*68*) dissolved steel anodically and collected the residue in dissolved oxygen-free water. The residue containing carbon and carbides was recovered by filtration and ground with glycerol; an aliquot was filtered on asbestos, washed, dried, and the carbon determined by combustion. Another aliquot was treated with hydrochloric acid to decompose carbides and the remaining free carbon was determined by combustion. The difference in the two values was considered carbide carbon.

Magnesium. Magnesium has assumed importance as an addition agent for iron. Methods for its determination are required in many laboratories. Bryan, Nahstoll, and Veldhuis (*11*) utilized cast $^3/_{16}$-inch rod electrodes as specimens for the determination of magnesium spectroscopically. Spark excitation is used. A range of from 0.005 to 0.15% magnesium in cast iron can be handled. Rozsa (*70*) also utilized the spectrograph for the determination. LaRochelle and Fournier (*45*) and Yarne and Sobers (*88*), respectively, described procedures for determining magnesium after separation of the iron with the mercury cathode and with an ether separation.

Molybdenum. Ellis and Olson (*20*) utilized acetone in place of stannous chloride to reduce molybdenum and the conventional yellow-amber color of the thiocyanate complex as a basis for

the quantitative determination of molybdenum. Acetone is said to increase sensitivity and eliminate fading of the color. Iron, which interferes, is removed by precipitation with ammonium hydroxide. Zaichikova (*89*) devised a procedure in which thiourea is substituted for the stannous chloride. Usatenko and Datsenko (*84*) separated molybdenum from a hydrochloric acid solution by adsorption on a cation exchange resin. The molybdenum is removed from the resin with an alkaline wash, reduced in acid solution with a bismuth amalgam, and titrated with permanganate.

Phosphorus. Bacon (*2*) found stannous chloride reduction unsatisfactory for use with the "molybdenum blue" method of determining phosphorus in steel and evolved a procedure in which he recommended use of ferrous sulfate as a reducing agent. The method, suitable for routine use, is said to give a reproducibility within ±0.001% of the phosphorus content. Greenberg, Weinberger, and Sawyer (*33*) investigated the effect of nitrite interference with the reduction of ammonium phosphomolybdate by stannous chloride in the molybdenum blue method. Sulfamic acid, when incorporated into the molybdate reagent, was found to control nitrite interference. Kassner and Ozier (*42*) modified the conventional alkali-molybdate method for the determination of phosphorus.

Potassium. Fast (*21*) developed a spectroscopic method for determining potassium in iron catalysts by distilling it quantitatively from a sample pellet during the initial arcing period and photographing the spectrum only during the period that the potassium was distilling out; this avoids interference from iron. An average deviation of from 5 to 10% of the true potassium content was obtained on repeated analyses. Sodium and lithium can be determined in the same manner.

Silicon. Dodero and Rambeaud (*19*) recommended solution of the sample in hydrochloric acid, followed by dehydration with a nitric acid–perchloric acid solution for determining silicon in 13% chromium-bearing steel. Petrova (*67*) utilized a mixture of sulfuric and nitric acids to dissolve high-chromium steels. He then added ammonium molybdate, reduced with stannous chloride, and finished by the molybdenum blue method. Murty and Sen (*58*) outlined an improved molybdenum blue method for determining silicon in steel.

Metallurgical Slags and Refractories. Gillis (*30*) recommended colorimetric and spectroscopic methods for the rapid analysis of slags and silicates. Kargin and Tkachenko (*41*) gave an account of a method for determining sulfur in blast furnace slag. Zuppann and Martin (*90*) outlined a method for the rapid analysis of slags by use of reflection and the spectrophotometer. Kassner and Ozier (*43*) utilized 8-hydroxyquinoline for the gravimetric determination of aluminum in ceramic materials.

Sulfur. Chernyi and Podoinikova (*16*) devised a micromethod for determining sulfur in pig iron, based upon evolution of the sulfur as hydrogen sulfide when the iron is fused with a mixture of oxalic acid and calcium metal.

Tin. The British Iron and Steel Research Association (*10*) recommended a method for determining tin in alloy steels based on solution of the steel in a nonoxidizing acid, separation of the unattacked complex alloy carbides, and precipitation of tin as the sulfide together with molybdenum sulfide as a "gatherer." The tin content of the sulfide precipitates is then obtained by the usual procedure.

Vanadium. Shaw (*71*) recommended an improved procedure for the indirect titration of vanadium following solution of the sample in 20% sulfuric acid, and oxidation of ferrous iron with concentrated nitric acid. Potassium permanganate (0.5%) is added in excess and the excess removed with sodium nitrite. Then sulfamic acid is used to decompose the surplus nitrite. An excess of standard ferrous ammonium sulfate is added, and the excess is titrated with standard potassium dichromate using barium diphenylamine sulfonate as an indicator.

Zirconium. Gump and Sherwood (*35*) made a systematic study of the factors involved in precipitation of zirconium and hafnium arsenates by formation of the arsenate ion within the solution. Their work on zirconyl chloride and hydroxide indicated optimum conditions which, if employed, were said to yield a precipitate of much better quality than the ordinary phosphate, hydroxide, or arsenate. The effect of other elements on the recommended method of precipitation was not indicated.

SUMMARY

The literature cited in this review does not include all published articles; it is intended to include those describing methods of specific application for routine analyses and also material which will be helpful in handling the unusual problems analytical laboratories so frequently encounter. It will be appreciated if important omissions are brought to the attention of the editors, so that they may be included in a future article.

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PETROLEUM

HARRY LEVIN, *The Texas Co., Beacon, N. Y.*

THIS review of progress in analysis in the field of petroleum takes into consideration, with a few exceptions, the literature for approximately one year from that covered in the previous review (*89*).

CRUDE OIL

Krause and Dystrup (*83*) employed the American Society for Testing Materials tetraethyllead extractor to remove salt from crude oil in benzene-acetone solution and determined the chloride in the extract by titration with silver nitrate to dichlorofluorescein indicator. Wenger and Ball (*161*) described an apparatus for analytical distillation of crude oil under artificial pressures to yield results at high altitudes like those obtained at sea level. Mojen (*112*) compared methods for determinating aromatics in crude oil. Moos (*114*), comparing solvent precipitation with adsorption for determination of asphalt in crude oil, found aluminum oxide satisfactory, silica gel and fuller's earth unsatisfactory. Frost and Stanfield (*41*) reported that specific gravity may be used to estimate the oil yield of shale in a particular area.

GAS

In a cooperative program of low temperature fractional distillation analysis in 47 laboratories, Miller (*106*) reported that in 95% of the laboratories the average deviation was less than 3% in analysis of C_3–C_5 alkanes. Starr *et al.* (*148*) established by mass spectrometry that much higher distillation rates than conventional may be employed in low temperature fractional distillation analysis without serious contamination of the fractions and showed that the contamination is due principally to the lower boiling constituents. Shively *et al.* (*141*) employed a boiling water–jacketed nickel catalyst to determine unsaturation of C_5 hydrocarbon mixtures, claiming greater speed and precision than with unheated catalyst. Hanna and Siggia (*54*) determined acetylene alkalimetrically by its reaction with potassium mercuric iodide and potassium hydroxide. Machemer (*93*) determined acetylene in liquid oxygen by evaporation through silica gel, from which the adsorbed hydrocarbons were subsequently swept by a stream of nitrogen into ammoniacal silver nitrate or copper sulfate in which the precipitated acetylide was determined by centrifuging; as little as 2 p.p.m. were detected.

Stroupe (*150*) described infrared methods for routine determination of small amounts of ethane, propane, *n*-butane, and isobutane in natural and purified methane streams. Martin (*101*) discussed requirements and applications of nondispersion-type infrared analyzers for plant streams of hydrocarbons. Wherry and Crawford (*162*) discussed requirements in instruments for continuous determination of components in plant streams of hydrocarbon mixtures and described actual installations for C_4 hydrocarbons from a furfural extractive distillation unit, isobutane in alkylation streams, and methane in other plant gas streams. O'Neal (*120*) combined infrared and mass spectrometric procedures to analyze C_1 to C_4 paraffin-olefin hydrocarbon mixtures, retaining the advantages of infrared for distinguishing butenes and those of mass spectrometry for other light hydrocarbons.

Shepherd (*140*) reported the results of cooperative analyses by 51 laboratories, comparing conventional volumetric chemical methods with mass spectrometry on a sample of carbureted water gas. Tickner and Lossing (*153*) used the mass spectrometer to determine vapor pressure of hydrocarbons of low vapor pressure;

the more conventional methods are subject to large errors due to traces of volatile impurities, whereas analysis by the mass spectrometer provides information for correcting for the partial pressures of the interfering impurities. Shepherd (*139*) reported the results of cooperative American Society for Testing Materials investigation of two volumetric absorption-combustion methods on natural gas. One measured contraction on combustion and resulting carbon dioxide; the other measured, in addition, the oxygen consumed. The second method proved more accurate. Nürenberg and Williams (*117*) described an apparatus for analyzing less than 1 ml. of a binary gaseous mixture, claiming measurements reproducible to ±0.04%.

Laitinen *et al.* (*84*) described a dropping mercury electrode null potential procedure for traces of oxygen in gas, claiming a sensitivity of 0.01% of oxygen. Thomas *et al.* (*152*) described apparatus and procedure for determining carbon monoxide in gases which contain olefins; the sample is separated into condensable and noncondensable gases by low temperature evaporation and the latter are subjected to Orsat analysis with acid cuprous chloride and cuprous sulfate-2-naphthol. Olefins interfere with more conventional methods for carbon monoxide. Weaver and Riley (*160*) determined moisture in gas by change in electrical conductivity of a phosphoric acid gelatin film between metallic electrodes. Claiborne and Fuqua (*17*) found analysis by infrared specially suited to the determination of small amounts of dimethyl ether in mixtures of methyl chloride, isobutylene, and isobutane. Fritz (*40*) employed a solution of perchloric acid in glacial acetic acid to titrate organic bases to methyl violet end point or potentiometrically, recommending the procedure for amines in polymerization feed stocks without the necessity of first evaporating the solvent. Miller (*107*) reviewed the physical and chemical methods of gas analysis in the literature of 1947, omitting spectroscopic methods.

GASOLINE

Dinneen *et al.* (*26*) compared bromine number, nitrogen tetroxide absorption, and silica gel adsorption for determining olefins and concluded that bromine number is unreliable as a measure of olefins in shale naphthas. Bricker and Roberts (*12*) detected and determined terminal unsaturation by oxidation with permanganate to form glycols, which were then split by periodic acid, followed by determination of formaldehyde equivalent to those double bonds. Lee *et al.* (*86*) employed iodine monochloride to determine unsaturation by a procedure which takes advantage of more rapid addition to original olefin than to decomposition products of the addition product. The method gave good results for diisobutylene and copolymers. Phillips and Wake (*123*) used iodine to determine unsaturation by a microprocedure on 6 mg. of sample. Microhydrogenation was employed by Ogg and Cooper (*119*) in a special apparatus and procedure, to determine unsaturation of fatty liquids. Braae (*10*) determined unsaturation by mercury-catalyzed addition of bromine to an electrometric end point, claiming that substitution and oxidation reactions are minimized, and that the method is hence suitable for easily substituted compounds such as hydrocarbons.

Newell (*116*) employed ultraviolet absorption to determine small amounts of styrene in polystyrene, claiming an accuracy of 0.05%. Pozdeeva and Stromberg (*126*) determined styrene in a crude product by a polarographic procedure in which the supporting electrolyte was ethyl alcohol solution of butyl nitrogen iodide. Marquardt and Luce (*99*) determined terminal unsaturation in olefinic compounds by reaction with mercuric acetate and titration of the acid liberated equivalent to the double bonds. Skoog and DuBois (*143*) determined indene in hydrocarbon mixtures by the color of the condensation product with benzaldehyde in the presence of alkali. Wilson (*168*) made a critical review of bromination methods for determining unsaturation of gasoline and considered the effect of peroxides on the results. He found that in the application of Kaufmann's reagent to hydrocarbons, substitution as well as addition occurred, but the bromine consumed approached that for complete addition, and concluded that addition and substitution in the same molecule are mutually exclusive. Ioffe (*67*) substituted a Pulfrich for an Abbé refractometer in the determination of aromatic hydrocarbons by dispersion and thereby reduced the absolute error from 1 to 2 to 0.3%. Forziati (*33*) reported precise refractive indices and specific dispersions for 60 hydrocarbons at 7 wave lengths.

A method for complete analysis of a mixture of C_7 and C_8 naphthenes was described by Bell (*5*), who combined infrared absorption with distillation and percolation methods; included are data for normal paraffins, isoparaffins, and naphthenes boiling up to 270° F. The approximate isoparaffin content of gasoline was determined by Funasaka (*42*) with antimony pentachloride reagent. The "tin point"—the dissolution temperature for tin tetraiodide in hydrocarbons—was used by Ketslakh *et al.* (*76*) to analyze hydrocarbon mixtures. Kramers and Broeder (*82*) considered thermal diffusion effect, multiplied, as a method for analysis of hydrocarbons.

Smith *et al.* (*145*) determined tetraethyllead in gasoline by reaction with alcoholic silver nitrate to produce a colloidal silver suspension whose intensity was determined with a photoelectric colorimeter; results within A.S.T.M. tolerances in 10 minutes are claimed. Iodine monochloride was used by Jahr (*69*) for the rapid determination of tetraethyllead in gasoline. The method is based on formation of diethyl lead dichloride, which ionizes, and determination of the chlorine ions. Hansen *et al.* (*56*) determined tetraethyllead in gasoline polarographically after dissolving the sample in Cellosolve containing hydrogen chloride; peroxides and unsaturates interfere. Offutt and Sorg (*118*) described a direct reading polarograph for the determination of tetraethyllead in gasoline. The instrument was applied to the acid extract of the sample, with antimony as pilot ion.

Hughes and Hochgesang (*66*) employed x-ray absorption to determine tetraethyllead in gasoline and made comparisons with chemical and polarographic methods. Calingaert *et al.* (*15*) used x-ray absorption, claimed an accuracy of 0.01 ml. of tetraethyllead per gallon when the base stock is available, and discussed methods for eliminating the interference of sulfur and halogens. Birks *et al.* (*9*) employed x-ray fluorescence, claiming independence from base stock or the composition of Ethyl fluid. Liebhafsky and Winslow (*91*) reviewed the principles of x-ray absorption measurements and emphasized the application to tetraethyllead blending operations. Vollmar *et al.* (*158*) described x-ray absorption methods for determining sulfur, tetraethyllead, and metallic additives in petroleum products. All claimed advantages in speed for the x-ray methods over more conventional procedures.

Mass spectra for ten C_5H_8 isomers were presented by Mohler *et al.* (*110*), who (*111*) also presented the mass spectra for 35 nonanes and discussed relationships between spectra and molecular structure. Friedel *et al.* (*38*) described a method for accurate measurement of the minute amounts of liquid samples employed in mass spectrometric analysis.

Fink *et al.* (*32*) discussed the effects of variables on the efficiency of separation of complex hydrocarbon mixtures by chromatographic adsorption and reported the conditions for maximum sharpness of separation. Godlewicz (*47*) employed an indicator on silica gel to produce a visible reaction zone with adsorbed aromatic hydrocarbons; trinitrobenzene proved a very satisfactory indicator. Mair (*96*) reviewed the development of the adsorption process (A.P.I. Research Project 6) for the analysis of hydrocarbons. Fink *et al.* (*31*) extended the analytical adsorption technique for the separation of cracked gasolines into classes of hydrocarbons to quantities sufficient to permit engine evaluation and studied the variables involved in this extension of the analytical procedure. Glasgow *et al.* (*45*), working in the opposite direction, described a micro adsorption column for 1

ml. of sample, in which the percolate from the column falls directly on the refractometer prism in the determination of aromatic hydrocarbons.

Rampton (*128*) combined distillation of original and dearomatized samples with ultraviolet absorption, silica gel adsorption and refractometry in the determination of six-membered naphthenes. Vaughn and Stearn (*156*) determined the composition of isomeric xylene mixtures by ultraviolet absorption measurements at four wave lengths and claimed accuracy within 1%. They obviated errors, due to deviations from Beer's law, by their method of plotting differences in absorption, and claimed general simplification over methods requiring measurement of extinction coefficients. Seidman (*136*) employed infrared absorption to determine Decalin in hydrocarbon mixtures, using a base-line technique to eliminate interference by other compounds. Coggeshall (*19*) reviewed the utility and limitations of infrared, Raman, ultraviolet, microwave, and mass spectrometric procedures for the analysis of hydrocarbons.

Buchanan *et al.* (*13*) described a photocolorimetric method for dye in aviation gasoline, claiming an accuracy of 0.1 mg. of dye per gallon. Hamence (*53*) described a paper chromatographic qualitative test for diphenylamine in gasoline. Glessner *et al.* (*46*) employed ultraviolet absorption to determine U.O.P. oxidation inhibitor No. 5; gasoline for background correction was obtained by eliminating inhibitor by agitation with acidified water. Pleeth (*125*) compared the magnesium nitride, calcium carbide, and Fischer methods for determining water in gasoline and concluded that the latter was most reliable. A "micro" method for determining knock characteristics of motor fuels was described by Alexander and Pfeiffer (*1*), who employed the customary knock engine on 20-ml. samples of fuel with a standard deviation of 1 octane number.

KEROSENE AND HEAVIER FUELS

Clerc *et al.* (*18*) compared three chromatographic methods of analysis in a study of straight-run and catalytically cracked gas oils and obtained satisfactory separation into saturates, monocyclic aromatics, dicyclic aromatics, and tricyclic aromatics. Hazlett *et al.* (*58*) determined the anthracene content of crude anthracene by ultraviolet spectrophotometry, claiming (*59*) 1% accuracy and greater convenience than chemical methods. The method was also used to analyze mixtures of anthracene, phenanthrene, and carbazole. McGovern and Anderson (*92*) determined thianaphthene in naphthalene by ultraviolet absorption at 297 mμ. Smith *et al.* (*144*) employed synthetic magnesium silicate in an adsorption procedure to separate shale oil distillates into a hydrocarbon fraction and one which was principally nitrogen and other heterocyclic compounds. Dinneen *et al.* (*27*) described adsorption analysis of small samples of shale oil distillates, with modifications in procedure for samples which showed poor separation.

Jackson (*68*) modified the A.S.T.M. apparatus for sediment in fuel oil by extraction, by incorporating a water trap to eliminate interference from this constituent. LeRosen and Wiley (*87*) determined pyridine and its homologs in hydrocarbons by extracting the base with dilute phosphoric acid and applying ultraviolet absorption spectrophotometry to this aqueous solution. Friedman *et al.* (*39*) employed beta-radiation, with Geiger-Müller counter outside the pipe, to determine water in fuel flowing through it.

LUBRICATING OIL

Grabe (*50*) estimated tar oil adulteration in mineral lubricating oil by its greater solubility in furfural. Williams (*167*) employed aluminum oxide in a chromatographic method to determine unsaponifiable matter in fatty oils, obtaining excellent results on blends of linseed oil with small amounts of mineral oil. Sandkühler (*131*) determined small amounts of lubricating oil in engine condensates by retention analysis with aniline dyes on filter paper. Beaven *et al.* (*3*) applied silica gel chromatography for the analytical separation of aromatic and saturated compounds in petroleum products of the lubricating oil range. Mills (*108*) modified the method of Lipkin for determining aromatic hydrocarbons in lubricating oil. The oil in *n*-pentane solution was percolated through a tube containing silica gel as upper layer and clay as lower layer, the effluent was stripped of pentane, the evaporation residue was weighed, and the aromatic hydrocarbons were determined by difference.

Conrad and Johnson (*20*) determined metals characteristic of additives in lubricating oil by a flame photometric procedure in which a solution of the sample in an organic solvent was atomized into the flame, avoiding the necessity for ashing the sample. Good reproducibility was shown for oils containing alkali and alkaline earth additives. Gassmann and O'Neill (*44*) determined phosphorus in lubricating oil by an emission spectrometric method employing a porous cup electrode, and claimed superiority over the quenched electrode procedure.

Ferguson (*30*) reported the results of cooperative A.S.T.M. investigation of a single phase titration procedure for determining acidic and basic characteristics of lubricating oils. Microtechniques employed in the analysis of sludges and deposits in lubricating systems were discussed, with actual cases, by Wiberley and Rather (*164*). Small amounts of furfural in oil were determined by Javes (*70*) by a colorimetric method based on reaction with 2,4-dinitrophenylhydrazine on filter paper. Malyugina and Korshunov (*98*) used the polarograph to determine furfural, claiming a relative error of 3%.

A multicolumn countercurrent molecular still was described by Madorsky (*94*). The chromaticities and daylight transmittances of petroleum products were studied by Judd *et al.* (*72*), on the basis of which they made recommendations for relocating the A.S.T.M. Union colorimeter scale to reduce errors in color grading petroleum products. Roberts and Levin (*130*), employing a special distillation unit to exclude atmospheric moisture, determined small amounts of water in oils, greases, and deposits by azeotropic distillation and titration of the distillate with Karl Fischer reagent. Hanna and Johnson (*55*) determined water in hydrocarbons by extraction with dry ethylene glycol and titration of the extract with Fischer reagent. Denton *et al.* (*24*) determined tetramethyldiaminodiphenylmethane (inhibitor) in oils spectrophotometrically after reaction with nitrous acid to produce yellow *p*-nitrodimethylaniline; dimethylaniline interferes.

ASPHALT

Mojen (*113*) determined paraffin in asphaltic products by adsorbing the sample on fuller's earth, extracting the nonasphaltic constituents with naphtha, removing resins from the nonasphaltic constituents with alcohol-ether, and isolating the paraffin by the Holde procedure.

SPECIALTIES

Layton (*85*) determined oil in petroleum wax by chilling the sample with methyl ethyl ketone to −25° F., filtering, reacting an aliquot of the filtrate with sodium bisulfite in a calibrated test bottle, and observing the volume of oil which separates on centrifuging. Good agreement with the A.S.T.M. method is claimed. Dietz *et al.* (*25*) determined oil in petroleum wax by ultraviolet absorption after establishing a working curve from oil content determined by the A.S.T.M. method. Results within 0.1% oil are claimed for samples containing up to 7%, 0.5 gram of sample being adequate. Zimmerschied *et al.* (*173*) determined normal alkanes in hydrocarbons of more than 13 carbon atoms by adduct formation with urea, decomposition of the adduct with water, and extraction of the liberated hydrocarbon with ether. Marshall (*100*) studied the methods for determining melting

point of wax, explained the reasons for divergences in results, and concluded that high melting microcrystalline waxes are subject to viscosity hysteresis at the melting point. Wallin (*159*) determined surface-active agents, including sulfonated petroleum, by reaction with fuchsin, which produces a color complex soluble in chloroform; the intensity of the solution is measured instrumentally. Friedel *et al.* (*37*) used infrared absorption analysis to determine qualitative and quantitative composition of mixtures of phenol, cresols, xylenols, and ethylphenols in mixtures derived from coal hydrogenation oil. Willard and Wooten (*166*) determined *o*-phenylphenol and *o-tert*-butylphenol colorimetrically after formation of their Aristols.

Golumbic (*49*) applied the Craig countercurrent distribution technique to the analysis of the complex phenolic mixture obtained from coal hydrogenation oil and found partial or complete separation among members of each group; isomeric phenols separate in order of decreasing acid strength and a relationship exists between partition coefficients and ionization constants of phenols. Treumann and Wall (*154*) employed infrared absorption to determine 1,2- addition in polymers and copolymers of butadiene. Francis (*35*) determined diglycols in monoglycols by eliminating the latter with periodic acid and distillation, and oxidizing the diglycols with potassium dichromate, whose excess he determined polarographically.

POLLUTION

Hubbard (*64*) determined benzene and its homologs in air with sulfuric acid formaldehyde reagent on silica gel, the length of stain being a measure of concentration. Heros (*61*) determined aromatic hydrocarbons in air by absorbing them in cold alcohol and making ultraviolet absorption measurements, at three wave lengths, on the solution. Vasserberg (*155*) determined hydrocarbons in air by titrating the carbon dioxide produced on combustion in a portable apparatus. Magill *et al.* (*95*), using 1 cubic foot of sample, determined as little as 0.05 p.p.m. of sulfur in air by a paper spot test based on formation of thallium polysulfide by sulfur and thallous acetate upon treatment with hydrogen sulfide. Gandolfo (*43*) employed filter paper impregnated with ammoniacal zinc nitroprusside to detect and determine small quantities of sulfur dioxide in gases. Kozlyawva (*81*) determined small amounts of sulfur dioxide in air colorimetrically with fuchsin-formaldehyde reagent. Katz (*75*) employed starch-iodine reagent for continuous determination of sulfur dioxide in air.

Shepherd (*138*) detected and determined carbon monoxide in air colorimetrically by passage over silica gel impregnated with ammonium molybdate and palladium sulfate, with a reproducibility of 2 p.p.m. in concentrations up to 100 p.p.m. The indicator changes from canary yellow through emerald green, blue green, and finally dark blue. Cahen and Letort (*14*) determined small amounts of carbon monoxide in air by combustion over platinized glass, absorbing and weighing the carbon dioxide which is produced.

Foyer (*34*) and Beerstecher (*4*) described chemical methods for determining small amounts of cyanide in air. Martin (*102*) determined phenol in water by the red color produced upon reaction with 4-aminoantipyrene in presence of potassium fluorocyanide. Zhitkova and Kut'in (*172*) determined acetylene in air by the color produced with a special copper nitrate reagent, whose preparation they described. Berton (*8*) employed ultraviolet absorption to determine minute amounts of furfural in air. Khlopin and Litvinova (*77*) determined tetraethyllead in air by absorption in a castor oil solution of iodine and methyl alcohol, chemical conversion to the inorganic form, and completion polarographically.

ELEMENTS

Murray and Plagge (*115*) determined the metals in gas and crude oils by ignition with pure silica or alumina and spectrographic analysis of the ash. Wrightson (*170*) determined traces of iron, nickel, and vanadium in petroleum oils by ashing, dissolving in potassium bisulfate, and analyzing the aqueous solution of the mixture by spectrophotometric methods; she employed 2,2′-bipyridine for iron, dimethylglyoxime for nickel, and diphenylbenzidine for vanadium. Karchmer (*73*) determined vanadium and titanium in petroleum ash colorimetrically by their peroxide complexes. Katchenkov (*74*) reported over 30 elements in petroleum ash and related the relative concentrations of the metals to geological formation and age. Carlson and Gunn (*16*) employed the cathode layer principle with added internal standards in a spectrometric method for determining trace metals in oils with which the carbon electrodes were impregnated. Susanina (*151*) determined iron in used oil, colorimetrically, after treating its ash with sodium salicylate.

Lescher (*88*) and Young (*171*) described a combustion method for carbon and hydrogen with special provision for controlling the vaporization of liquid samples. Berret and Poirier (*7*) described a modification of the Unterzaucher method for determining oxygen in organic substances and included a special procedure for freeing the nitrogen of oxygen. Carbon monoxide was oxidized by passage over silica gel impregnated with iodine pentoxide and sulfuric acid, and the carbon dioxide was determined gravimetrically. In another modification of the Unterzaucher method Deinum and Schouten (*23*) oxidized the carbon monoxide with mercuric oxide, absorbed the resulting carbon dioxide in barium hydroxide, and titrated the excess of the latter. The nitrogen was purified with copper in ammoniacal ammonium chloride. Maylott and Lewis (*104*) compared the ter Meulen, Liebig, and Unterzaucher methods for oxygen in organic compounds and discussed the limitations of each of these procedures.

Hale *et al.* (*51*), concerned with very low concentrations of combined nitrogen in petroleum, described a semimicro Kjeldahl apparatus and procedure in which the ammonia was determined spectrophotometrically after nesslerization. To determine bromine and chlorine in gasoline, Pecherer *et al.* (*122*) decomposed the ethylene halides with disodium biphenyl, whose preparation they described. Milner (*109*) determined fluorine in fluorinated organic compounds by burning in oxygen and water vapor in a platinum tube and titrating the liberated hydrogen fluoride. Simmons and Robertson (*142*) determined phosphorus in organic compounds by the molybdiphosphate alkalimetric method, after conversion into ionized orthophosphate by reaction with hydriodic acid or complete wet oxidation in the presence of a molybdenum catalyst.

SULFUR AND ITS COMPOUNDS

Wilson and Straw (*169*) described a rapid method for determining low concentrations of sulfur in products varying from gasoline to sperm oil. The sample was burned in a rapid current of air in a vertical tube packed with vanadium pentoxide on alumina. Kirsten (*79*) determined sulfur by burning the sample in a stream of oxygen, reducing the products of combustion to hydrogen sulfide in a hydrogen flame, absorbing in alkaline solution, and determining with hypochlorite. The method is also applicable to inorganic substances by covering them with phosphorus pentoxide in the combustion step to expel sulfur.

Holeton and Linch (*63*) described improvements in apparatus and procedure for determining traces of sulfur in hydrocarbons by combustion with air in a heated tube. They relate to atomizer design, absorption of oxides of sulfur, and stabilization of the colloidal barium sulfate for nephelometric determination. Kirshenbaum and Grosse (*78*) extended their isotopic method to include the determination of sulfur, using $S^{34}O_2$ as tracer. Hall (*52*) described a polarographic method for determining elementary sulfur in petroleum fractions, claiming an accuracy of 2% in samples that contain up to 100 p.p.m. Berk and Burdick (*6*) found that the error in determining sulfur trioxide in the presence

of sulfur dioxide, by absorption in alkali containing benzyl alcohol as oxidation inhibitor, is due to traces of copper oxide which promotes oxidation of sulfur dioxide, and that inhibition is improved if a small amount of benzaldehyde and *p*-aminophenol hydrochloride is added to the absorbing liquid.

Atkin (*2*) described a colorimetric method for determining sulfur dioxide in gases resulting from catalytic sulfuric acid manufacture, based on the reaction between sulfur dioxide and fuchsin formaldehyde reagent. Price (*127*) determined sulfuric acid in refinery "black" acid by its electrical conductivity. Strafford *et al.* (*149*) attributed errors in mercaptan content by amperometric titration to air dissolved in the reagents. Picon (*124*) described a procedure for separating, purifying, and determining thiophene homologs from shale. Goehring *et al.* (*48*) outlined a scheme of analysis for determining individual polythionates in a mixture. Haresnape *et al.* (*57*) employed chromatographic adsorption on silica gel to separate organic sulfur compounds from each other. Schreiber (*135*) presented the infrared spectra of a large number of organic sulfur compounds. Seyfried (*137*) reported the progress of American Petroleum Institute Project 48, the aim of which is identification and determination of sulfur compounds in crude oil and development of apparatus and methods to accomplish it.

CATALYSTS

Fast (*29*) found that during the initial arcing period alkali in an iron catalyst distills quantitatively from the sample pellet, and by photographing the spectrum during this period potassium, sodium, and lithium can be determined without interference from iron. Free and combined iron in cracking catalysts were determined colorimetrically with *o*-phenanthroline by Snyder and Clark (*146*) by determining total iron after digestion with hydrochloric acid and free iron after treating the catalyst with a neutral copper sulfate solution and removing the excess copper by displacement with pure aluminum. Vogel (*157*) determined metallic iron in presence of its oxides by dissolving the former in a solution of bromine in ethyl alcohol. Korzh (*80*) reported that sodium may be determined in clays without spectroscopic apparatus by observing the time required for a sudden increase in voltage when the sample mixed with calcium sulfate is arced. Calcium sulfate releases the sodium and disappearance of sodium lines always occurs at the same arc voltage; with the appearance of calcium the voltage suddenly increases.

Hofer and Cohn (*62*) discussed the application of the magnetic balance to the ferromagnetic phases in Fischer-Tropsch catalysts. Schmitkons (*134*) described the application of the Leco carbon determinator to the determination of carbon on cracking catalysts, claiming operating time of 10 minutes and error of less than 1%. Wilchinsky (*165*) described a system of analysis for determining the morphological features of powder particles, the deductions being made from the manner in which quantitative composition for a chosen component varies with particle size. Milberger *et al.* (*105*) described a procedure for determining water in hydrogen chloride with Karl Fischer reagent; C_4 hydrocarbons did not interfere.

MISCELLANEOUS

Orchin *et al.* (*121*) discussed the possibilities of applying a mixture of high molecular weight hydrocarbons containing combined hydrogen and combined deuterium to the determination of deuterium in water. Cooper and Medcalf (*21*) described an internal electric immersion heater for Engler-type distillations of many materials, including petroleum distillates, and claimed reduction in fire hazard and improvement in efficiency. Santora (*133*) described an apparatus for determining vapor pressure of small samples of hydrocarbons and showed good agreement with the A.S.T.M. Reid vapor pressure method. Matteson (*103*) modified the differential vapor pressure thermometer of Menzies and used it with a special ebulliometer to determine molecular weights up to 1000 on as little as 100 mg. of hydrocarbon sample in approximately 25 minutes; the boiling point of the sample must be at least 150° C. higher than that of the solvent.

Rather *et al.* (*129*) discussed the training of chemists for microchemical work in the petroleum industry. Levin *et al.* (*90*) described microtechniques for determining pour point of lubricating oil, vapor pressure of gasoline, and titer of fatty acids. Sommer and Wear (*147*) described microprocedures for determining gravity, viscosity, and distillation of petroleum products.

Curtis *et al.* (*22*) discussed the problem of measuring evaporation rates of solvents and concluded that atmospheric humidity, nature of surfaces from which liquids are evaporated, chemical constitution, and thickness of the films are important factors. A continuous recording refractometer for industrial control was described by Jones *et al.* (*71*), who claimed a sensitivity of 0.00005. Sankin *et al.* (*132*) presented an equation for conversion of refractive dispersion to different wave lengths, for hydrocarbon and nonhydrocarbon liquids. Enverard and Hurley (*28*) determined surface tension of viscous materials by measuring the length of an air bubble of given volume in a horizontal tube of known diameter. Heigl *et al.* (*60*) described a recording Raman spectrometer for analysis of hydrocarbon mixtures. Braun *et al.* (*11*) gave the Raman spectra of 119 different compounds. White *et al.* (*163*) described a recording infrared spectrometer for continuous determination of six components in a stream. Hughes *et al.* (*65*) discussed the application of emission spectroscopy to the quantitative analysis of samples in a petroleum laboratory. Freund (*36*) determined the adhesive properties of mineral oil lubricant to solid surfaces by determining its behavior under centrifugation. Majumdar (*97*) determined carbonaceous adulterants of graphite, such as charcoal, coke, and coal, by burning them at about 700° C. The graphitic carbon was subsequently determined by ignition above 900° C.

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Pharmaceuticals and Natural Drugs

WAYNE W. HILTY AND MAX M. MARSH, *Eli Lilly and Co., Indianapolis, Ind.*

THE past year has seen a considerable increase in the number of foreign publications pertaining to the field of pharmaceutical analysis. In addition, the steadily increasing scopes of the analytical methods employed in the examination of preparations of pharmaceutical interest have uncovered avenues of investigation previously not seriously considered from the viewpoint of drug investigations.

In order to permit better classification of the various procedures described in this review, two new groups have been established—hormones and related substances, and proteins and amino acids. The recent increase in interest in hormones, particularly cortisone, ACTH, and related compounds, appears to warrant such separation, while the protein and amino acid grouping is an outgrowth of an increasing number of publications in this category which tend to overcrowd the general groups.

Again, the authors wish to emphasize that the review presented here is by no means a critical one in the sense that omissions signify worthless procedures; it is true, however, that the publications included are those which, in the authors' opinions, offer the greatest possibilities for application in the field of analysis of pharmaceuticals and natural drugs.

As in the previous annual reviews, the methods discussed have been divided into three broad classes: Chemical Methods, Physicochemical Methods, and Physical Methods.

CHEMICAL METHODS

ALKALOIDS AND RELATED SUBSTANCES

Chromatographic procedures for the separation of adrenaline from mixtures and the subsequent elution with dilute acid have been reported by Bjorling and Hellberg (*13*), as have identification and characterizing procedures for alkaloids and other vegetable products by simple color tests (*83*). Identification tests for cocaine (*129*) and cocaine in the presence of procaine and other aminobenzoic anesthetics (*55*) have been reported. The determination of codeine in sirups may be made by precipitating an extract of the codeine with silicotungstic acid (*53*). Digitoxin may be fractionated from seemingly homogenized preparations by adsorbing on alumina (*123*). Epinephrine (*15*) has been the subject of a critical study of various oxidation reactions. Organic bases (*45*) such as brucine may be titrated with perchloric acid in dioxane, using modified methyl orange and methyl red indicators.

Colored products (*7*) with papaverine, thebaine, acetanilide, saponin, ephedrine salol, homatropine, and benzidine may be obtained when these are treated with barium peroxide in sulfuric acid. According to Wankmuller (*159*), procaine may be determined by bromination.

A potentiometric titration method for phenobarbital (*104*) is described, which the authors claim gives results more accurate than those obtained with the U.S.P. procedure. Theophylline (*120*) may be determined on a semimicro volumetric scale in mixtures of theobromine, theophylline, and caffeine. Titrations of organic bases (*46*) in nonaqueous solvents have proved satisfactory for the determination of pyridine, aniline, benzylamine, and brucine. Keenan has reported the optical characteristics of brucine sulfate (*78*).

Curare alkaloids (*134*) may be separated by paper chromatography and then identified by ultraviolet fluorescence or their color reactions with ceric sulfate. A chemical assay for aconite (*111*) involving ether extraction from the crude drug and determination of total alkaloids followed by saponification, acidification, and final steam distillation of the benzoic and acetic acids formed from aconitine is reported by Muhlemann and Weil.

An article published by Edmundson and Wilkins (*36*) suggests that chlorophyll may be used in the alkaloidal assay of solanaceous drugs to prevent emulsification.

ANTIBIOTICS

A modified rapid development technique for the microchromatographic assay of various types of penicillin has been published by Glister and Grainger (*50*). The problem arising out of the quantitative interpretation is discussed, and it is indicated how, by using developed mixtures of pure penicillin standards, the results are rendered more representative. A kieselguhr–sodium citrate (*30*) buffer partition chromatogram for separation of various penicillins has been described.

The potassium iodide concentration and its influence in the iodometric titration of penicillin were discussed by Canback *et al.* (*19*). A procedure for the determination of penicillin in penicillin beer which has been saturated with ammonium sulfate

has been described by Penau *et al.* (*119*). The penicillin is extracted with methyl ethyl ketone and then is determined iodometrically.

CHEMOTHERAPEUTIC AGENTS

A method for the estimation of the assay value and degree of substitution of sodium carboxymethylcellulose has been published by Conner and Eyler (*25*). The insoluble copper salt of the cellulose derivative is precipitated, treated, and weighed. The copper content of the precipitate is then determined iodometrically as a measure of the degree of substitution. The method is not recommended as a routine procedure because of the length of time involved. Average recoveries of 98% or more are claimed.

Sulfonamide compounds (*85*) may be titrated with alkali using thymolphthalein as the indicator. Srinivasan (*147*) has described the potentiometric titration of small amounts of procaine, sulfanilamide, and related compounds by using bromate-bromide reagent. Color tests for the determination of thiouracil (*106*) and some of its derivatives utilizing 10% copper sulfate have been published. It has also been reported that thiourea (*61*) may be quantitatively oxidized to urea by alkaline peroxide. The urea may then be determined from the ammonia produced by action of urease.

HORMONES AND RELATED SUBSTANCES

Identification tests for synthetic stilbene estrogens (*144*) and some color reactions of estrogenic hormones (*124*) have been described recently. A new color reaction of folliculin (*74*) with pyruvaldehyde and sulfuric acid has also appeared in the literature along with new color reactions for steroids (*121*).

PROTEINS AND AMINO ACIDS

α-Amino acids quantitatively evolve the ammonia of their α-amino groups by the action of peri-naphthindan-2,3,4-trione hydrate and its *m*-nitro derivative in an aqueous medium at pH 4.7. A simple and rapid semimicromethod (*110*) for the measurement of this evolved ammonia has been described. Amino acids may be precipitated from aqueous solutions by use of dibenzofuran-2-sulfonic acid (*165*).

The production of a fluorescent, red pigment tryptochrome has been described as a sensitive and selective test for free tryptophan and tryptamine (*42*).

The estimation of arginase (*158*) may be made by determining the urea formed by its action on arginine. Angiotonin (*63*) may be separated from crude mixtures by simple chromatographic procedures.

METALLIC IONS AND RELATED SUBSTANCES

A method for the volumetric determination of aluminum (*131*) in the presence of free acid and iron has been described by Ringbom. A microdiffusion method for the determination of calcium has been published (*112*), in which the calcium is precipitated as the oxalate and then dissolved in sulfuric acid and oxidized to carbon dioxide, which is absorbed by an excess of standard barium hydroxide. Calcium (*103*) may also be determined by complexation with excess disodium ethylenediamine tetraacetic acid. The solution is then buffered to pH 10 and the excess complexing agent is titrated with magnesium chloride using Eriochrome Black T indicator. Phenolphthalein (*161*) may be determined volumetrically by a method based on the iodination of phenolphthalein in alkaline solution and titration of the excess iodine with sodium thiosulfate.

What has been described as a new test for chlorine and bromine has been published by Milton (*109*). The test involves formation of cyanogen chloride by the reaction of sodium cyanide and chlorine to give sodium chloride and cyanogen chloride; the formation of a quaternary compound by cyanogen chloride and pyridine, or a derivative and condensation with aromatic amines, leads to ring fission and formation of intensely colored dianil derivatives.

Iodine (*167*) and bromine (*166*) in organic compounds may be determined by Carius iodometric microdetermination. Mercury (*108*) may be separated from copper by dissolving the sample in sulfuric acid and hydrogen peroxide. Mercury is then reduced to metal with stannous sulfate and distilled as the metal from the acid solution. A method for the determination of mercuric chloride with sodium hydroxide has been described by Silvertri (*141*). A modification of the sodium fusion method is the best method for the decomposition of organic compounds into inorganic ions and the subsequent detection of nitrogen as cyanide according to Campbell *et al.* (*18*).

Total phosphorus in organic phosphates (*142*) may be quantitatively converted to ionized orthophosphate by reaction with hydriodic acid or by destructive oxidation with a nitric, perchloric, and sulfuric acid mixture. The combustion train method for the determination of sulfur (*66*) has been refined by inclusion of an atomizer in the train, which permits precise control of the flow rate of the spray into the combustion chamber. Sulfur is estimated turbidimetrically as suspended barium sulfate. Accuracy of 5 to 10% and precision of ± 2 p.p.m. in the range of 2 to 100 p.p.m. is claimed.

VITAMINS

A method utilizing chromatographic separation for determining ascorbic acid and dehydroascorbic acid has been suggested by Mapson and Partridge (*97*). It has been reported that in alkaline solution, 1 mole of vitamin B_1 (*56*) reacts with 3 moles of iodine. After acidification, the residual iodine from an excess may be titrated with sodium thiosulfate using starch T.S. as the indicator. In the case of vitamin B_1 in pharmaceutical products, extraction of the thiamine hydrochloride is recommended before reaction with iodine.

A colorimetric method for calciferol (*122*), which depends upon the condensation with aldehydes, has been described and is reported to be sensitive in the range 250 to 1000 micrograms. Emmerie (*39*) has described the chromatographic separation of tocopherols using aluminum oxide activated at 106–108° C. The solvent consists of one volume of absolute ethyl alcohol plus 99 volumes of light petroleum.

Pankratz and Bandelin (*116*) have developed a gravimetric method for determining choline with ammonium reineckate. Auerbach (*6*) has published a note referring to a correction of these techniques for coprecipitated bases present in certain liver factors which are essentially acetone-insoluble.

GENERAL

Wilbur *et al.* (*170*) have described the technique in which thiobarbituric acid is used as a reagent to test for the oxidation of unsaturated fatty acids. Long-chain fatty acids (*69*) may be separated by using reverse phase partition chromatography.

Iodine numbers (*153*) may be determined by direct titration with bromine in glacial acetic acid. A color test for fructose (*105*), which is believed to have a sensitivity of 0.1 microgram per milliliter, has been reported. Paper partition chromatography is the basis for an analytical method for sucrose (*34*) which has a utilizable range of 10 to 350 micrograms.

Ketohexoses (*151*) may be identified by reaction with aminoguanidine sulfate and water to give reddish purple colors. Sensitivity appears to be limited to 50 micrograms. Methods for the determination of essential oils (*118*) involving distillation with ethylene glycol have been discussed by Patin and Vigneau. Sodium formaldehyde sulfoxylate, which is used in some allergenic extracts to prevent oxidation and discoloration, has been shown to interfere in the usual steam-distillation technique for the separation and estimation of phenol (*126*) which is usually contained in these biological materials. This interference may be removed by adding lead acetate to the distillation chamber.

The purity of propylene glycol (*107*) may be determined by using a test which depends on the critical solution temperature of propylene glycol and ether. With this test the presence of 0.1% of dipropylene glycol or similar amounts of water and ethyl alcohol in propylene glycol can be determined. Pertinent chemical and physical data relating to a series of polyethylene glycols (*139*) 200 to 10,000, inclusive, have been assembled and reported by Shaffer *et al.* A chromatographic technique for the separation of sugars (*68*) and their methylated derivatives has been reported which utilizes columns of powdered cellulose. The Karl Fischer method for the determination of water (*76*) has been modified by the addition of bromine which oxidizes the hydriodic acid formed to iodic acid, which is determined by iodometry.

PHYSICOCHEMICAL METHODS

ALKALOIDS AND RELATED SUBSTANCES

Fluorometric methods for the determination of adrenaline (*4, 37, 136*) have been described, with supporting data. Local anesthetics (*148*) such as procaine, panthesin, pantocaine, and nupercaine produce reineckate salts when they react with ammonium reineckate. The reaction has been made the basis for quantitative colorimetric methods for these preparations. This versatile reagent will also precipitate alkaloids (*8*) from an aqueous solution. The reineckate precipitate is dissolved in acetone and absorption at 525 mμ determined. The method is rather nonspecific and quaternary ammonium salts and heterocyclic amines will interfere. Ammonium reineckate reagent may also be used for the determination of antihistamines (*9*). Haley and Keenan (*58*) have listed microchemical tests and optical crystallographic properties of 12 new antihistamines.

Cocaine (*129*) will produce a strong purple color after nitration of the benzene ring. This reaction appears to be specific for cocaine. Methadone (*130*) may be determined colorimetrically with methyl ethyl ketone after nitration of the phenol radicals. The possibility of an indirect colorimetric method for the assay of ephedrine (*168*) has been suggested by Wickstrom. This author reports that ephedrine can be oxidized by periodic acid at room temperature to yield 1 mole each of acetaldehyde, benzaldehyde, and methylamine. It is claimed that the acetaldehyde formed may be distilled and determined colorimetrically.

A procedure for the estimation of morphine (*115*), in which a pink to red color is produced in the presence of ammonium hydroxide, copper sulfate, and hydrogen peroxide, has been reported. Esters of morphine and apomorphine are said to produce the same color, while dionine and codeine do not. A method for the determination of morphine (*33*) in opium is described in detail, in which the calcium extract is employed for a colorimetric determination using iodic acid and ferric chloride. The maximum absorption is determined at 4700 A. Methods have been described for the photometric determination of the opium alkaloids (*163*), codeine, thebaine, and narcotine. These methods, however, are satisfactory only for pure solutions of the respective alkaloids.

A new method of extraction and isolation of the alkaloids of ipecac (*88*) has been published, in which the extracted alkaloidal salts are adsorbed on a Florisil column. The alkaloids are then eluted with ammoniacal alcohol. Neuwald (*113*) reports that, contrary to previous reports, Beer's law is obeyed by the color formed from the Baljet reaction for digitoxin if the measurements are made at 500 mμ. Kennedy (*79*) has studied the modified Bell and Krantz method as applied to the assay of the three U.S.P. digitalis glycosides. The picrate reagent in tetraethyl ammonium hydroxide has been used to assay digoxin and lanatoside C as well as digitoxin. Optical densities are read at 495 mμ instead of 525 mμ with increased sensitivity. The accuracy is reported to be ±2%. Bell and Krantz (*11*) have also studied this reaction with sodium hydroxide and tetraethyl ammonium hydroxide. They verify a maximum absorption peak of 490 to 495 mμ and they believe that the specificity of the reaction has been underrated because the color developed appears to be due to a complex of the glycoside and picrate ion rather than to the picramate ion.

Mixtures of morphine, heroin, codeine, and barbiturates (*149*) may be separated by using a chromatographic technique with Florisil as the adsorbing agent. The adsorbed constituent is eluted and final determination made colorimetrically. Jindra and Pohorsky (*75*) have attempted to discuss the use of ion exchange resin IR4B for the separation of several alkaloids. A colorimetric method for the determination of physostigmine (*102*) has been described by Masse. Colchicine (*94*) may be determined colorimetrically when aldehydes and ketones are absent. Senna (*21*) may be evaluated fluorometrically, while neo-synephrine (*5*) may be determined colorimetrically in pharmaceutical products by coupling the drug with diazotized *p*-nitroaniline in borax buffer.

Procaine and related compounds (*147*) may be determined by direct titration with potassium bromate solution with a pair of polarized platinum electrodes. The method is reported to be simple and rapid and to yield results of high precision and accuracy. This method may be applicable to the sulfa drugs.

Washburn and Krueger (*162*) have proposed an infrared absorption procedure for the determination of aspirin, phenacetin, and caffeine.

ANTIBIOTICS

Fluorometric methods for the determination of aureomycin (*89, 137*) permit the estimation of 0.1 to 20 micrograms and yield good correlation with bacteriological assay. Chloromycetin (*12, 49*) may be determined colorimetrically after reduction, diazotization, and coupling. A spectrophotometric method for determination of streptomycin (*38*) based on steam distillation of the maltol formed by the alkaline pretreatment of the streptomycin and the use of ion exchange resin IRC50 (*31*) to separate the streptomycin have been described. Dihydrostreptomycin salts (*24*) may be assayed by oxidizing with periodic acid in a Kirk distillation apparatus, collecting the evolved formaldehyde in sodium bisulfite, and determining it with chromotropic acid reagent. Both streptomycin and dihydrostreptomycin (*16*) are said to give red colors with 1-naphthol and sodium hypobromite.

CHEMOTHERAPEUTIC AGENTS

Gentisic acid (*2*) forms a blue color when treated with iron perchloride. This reaction has been made the basis of a quantitative assay for this material.

Gentisic acid (*145*) may also be measured colorimetrically or by ultraviolet absorption at 320 mμ. Sulfonamides (*138*) may be determined by thermometric titration with hypochlorite. Thiosemicarbazone (*173*) may be determined colorimetrically. A method for the determination of propamidine (*154*) by means of pentacyanoammonioferrate has been suggested by Trought *et al.* All sulfonamides (*86*) official in the U. S. Pharmacopeia XIII may be titrated potentiometrically, using a platinum-calomel electrode pair. Cooling of the solution with ice is not necessary; the method appears to be somewhat more precise than the external starch-iodide indicator procedure.

METALLIC IONS AND RELATED SUBSTANCES

A polarographic method for aluminum based on the reduction of its complex with Pontachrome Violet SW at −0.5 volt versus the saturated calomel electrode has been reported by Willard and Dean (*171*). Phosphoric acid (*44*) may be determined by an indirect colorimetric assay.

Micromethods for the determination of potassium (*1, 80*), said to be sensitive in the range of 1 to 100 micrograms, have been reported.

Cobalt (*127*) may be detected with triphenylsulfonium bromide. Sensitivity is said to be about 0.05 microgram. Citrinin

has been reported to be a good inorganic analytical reagent for iron (*140*). Magnesium (*26*) may be titrated conductometrically in the presence of limited amounts of calcium. Sodium (*150*) may be determined colorimetrically after precipitation of the triple salt with uranium and zinc. A micromethod for the determination of sulfur (*67*) by means of the iodine-azide reaction has been reported by Holter and Løvtrup.

HORMONES AND RELATED SUBSTANCES

The identification of estrogens (*62*) by paper chromatography and a discussion of the groups involved in the Zimmerman and Kober reactions (*99*) have been reported. Estrogenic substances (*54*) in sulfuric acid may be measured by fluorometric procedure. Only a single estrogen in a particular vehicle may be measured. The method does not distinguish between estrogens. Traces of ferric ion in the Kober reagent for estrogens (*57*) increases the sensitivity. The diluted reagent still exhibits high sensitivity for β-estradiol, but not for α-estradiol. Equilin and equilenin (*10*) may be determined in the presence of estrone by reaction with dibromoquinonechloroimide.

Cortisone (*125*) and related 17,21-dihydroxy-20-ketosteroids may be determined colorimetrically by a procedure which appears to be specific for this group of steroids.

PROTEINS AND AMINO ACIDS

A rapid micromethod for the estimation of nonvolatile organic matter by dichromate oxidation has been reported by Johnson (*77*). Particular reference is made to proteins and carbohydrates. Cysteine and cystine (*82*) may be determined by argentometric amperometric titration procedures. Tryptophan (*51*) is reported to produce a green fluorescence with perchloric acid, and this reaction has been made the basis for a quantitative estimation. Copper salts of lysine (*3*) may be separated by chromatographic procedures and determined colorimetrically with iron ferrocyanide.

Blackburn and Robson describe a radioactive technique for the microestimation of α-amino acids (*14*) and its application to paper partition chromatograms.

VITAMINS

Trichloroacetic acid has been recommended as a color reagent for vitamin A (*114*). This reaction is reported to be more specific and less sensitive to water, and supposedly has a slower decrease in color intensity than the antimony trichloride reagent.

An assay which is said to be applicable to impure solutions of not less than 40 micrograms of B_{12} (*41*) in 2 ml. has been described by Fantes *et al.* The B_{12} is hydrolyzed in acid medium to a red acid which is esterified with octyl alcohol. The color then can be determined spectrophotometrically. The method does not differentiate between B_{12} and B_{12b}, and the authors report results with an error of ±5%. Ganguly (*47*) has reported a colorimetric method for the determination of pteroylglutamic acid and related compounds in liver extract concentrates. Folic acid (*160*) may be determined in preparations containing B complex vitamins, liver, and iron; the zinc-amalgam reductive cleavage method is used. When ferrous salts are present, sodium gluconate is added to prevent precipitation of ferrous iron.

Various methods for the determination of nicotinic acid (*23, 29, 71*) have been reported in the literature. Ciussa (*22*) reports that nicotinic acid and nicotinamide may be determined in the same solution.

Pantothenic acid (*27*), according to Crokaert, may be determined colorimetrically after hydrolysis to β-alanine. Panthenol and pantothenates (*174*) yield pantoyl lactone on acid hydrolysis. The hydroxamic acid formed by the reaction of the lactone with hydroxylamine exhibits a purple color in the presence of ferric ions. The authors of this paper have described optimum conditions for the reaction and color measurement of this product. They have utilized ion exchange columns to remove interfering ionic substances in the determination of panthenol. Pantothenates cannot be isolated in this material and must be measured in relatively pure solution.

Rutin (*128*) produces a yellow color with ammonium molybdate, which is proportional to rutin concentration. The color is light-stable and differs from the normal ammonia color.

GENERAL

The condensation with D-1-ribitylamino-2-amino-4,5-dimethylbenzene to produce riboflavin is utilized as the basis for a fluorometric method of determination of alloxan (*152*). The effect of various experimental conditions on rate of formation and yield of riboflavin is discussed.

A method for the microestimation of trimethylamine in *Chenopodium vulvaria* L. is described by Cromwell (*28*). The microestimation of citric acid (*164*) may be accomplished by modifying the colorimetric estimation for citric acid by replacing permanganate with vanadic acid. This gives increased simplicity and speed of operation and greater freedom from interference by other oxidizable substrates. Dihydroxyphenols (*172*) may be determined colorimetrically by iodination. Pyridine copper sulfate reagent may be used to determine fumaric acid (*100*). The reaction with benzaldehyde to produce intensely yellow colored 1-(α-hydroxybenzyl)-3-benzalindene is used as the basis for a spectrophotometric method for indene (*143*).

The modified diphenylamine procedure, which substitutes autoxidation of glucose in alkaline solution for the usual glucose fermentation, has been proposed for the determination of inulin (*92*). A semimicromethod for the estimation of lactose (*96*) alone and in the presence of other sugars has been described. Modifications are given for its application to solutions containing lactose in the presence of glucose and glycogen and in tissue extracts when other contaminating sugars are present. Linolenic acid (*32*) may be determined colorimetrically after it has been oxidized and heated with thiobarbituric acid. Malic acid (*73*) may be determined fluorometrically in the presence of succinate, fumarate, aspartate, butyrate, or malonate. Paper chromatography has been used as technique for the identification and estimation of purines, pyrimidines, and related substances (*98*). A method based on the fading effect of oxalate on the green ferric-Ferron complex is reported to permit a colorimetric estimation of oxalate (*17*). After bromination, uracil and cytosine reduce lithium arsenotungstate uric acid reagent. This has been made the basis for a colorimetric method for the estimation of uracil and cytosine (*146*). Water (*59*) may be determined in hydrocarbons by extraction with dry ethylene glycol and subsequent titration with Karl Fischer reagent. The determination of water (*72*) indirectly in vegetable drugs by the Karl Fischer method using polarized platinum electrodes is also reported.

The Folin colorimetric method for amino acids using 1,2-naphthoquinone-4-sodium sulfonate has been applied to histamine (*95*); this method has also received recognition in the U. S. Pharmacopeia XIV (*155*). A comparative assay procedure for volatile oils (*90*) has been proposed in which the sample reacts in tetrahydrofuran with lithium aluminum hydride. Excess lithium aluminum hydride is titrated potentiometrically and results are expressed in millicquivalents of the hydride utilized by the oil. A titrimetric method for determining functional groups in pharmaceutical products with lithium aluminum hydride has been published by Lintner *et al.* (*91*).

PHYSICAL METHODS

ALKALOIDS AND RELATED SUBSTANCES

Relatively pure solutions of alkaloids (*132*) and certain antihistamines (*101*) may be assayed by spectrophotometric measurement of the absorption of ultraviolet radiation. The physical characteristics of the 2-anthraquinone sulfonates of morphine and codeine (*43*) may prove useful as an identification technique. Simultaneous equations applied to the optical densities of quinine and cinchonine (*52*) at 316 and 348 mμ permit the estimation of

the amounts of these types of alkaloids present in a given solution. This technique has been successfully applied to extracts of cinchona bark. Small quantities of methadon (*70*) may be identified by determining the ultraviolet absorption spectrum of the methadon base. The optical properties, microchemical reaction, and x-ray diffraction data for methadon are also reported.

ANTIBIOTICS

Penicillin (*48*) may be determined by an ultraviolet absorption technique after extraction with chloroform from an aqueous solution at pH 2. The polarographic method of analysis for chloromycetin in pH 4 buffer has been described by Hess (*64*). The method agrees well with bioassay.

CHEMOTHERAPEUTIC AGENTS

Schack and Waxler (*133*) have reported an ultraviolet spectrophotometric method for the determination of theophylline and theobromine.

HORMONES AND RELATED SUBSTANCES

Schneider reports the isolation and spectrophotometric determination of cortisone (*135*). Progesterone (*60*) may be separated by using paper chromatographic techniques with final ultraviolet measurement as a means of estimating potency. Estrogenic hormones (*40*) may be separated by the use of a suitable neutral solvent system in a 24-transfer countercurrent distribution instrument. The three estrogens may be separately identified by a physical constant and quantitatively determined.

Infrared spectrophotometry has been the basis of measuring α-estradiol and other estrogenic diols (*20*). Chromatographic separation has been utilized to permit the determination of background absorption of the components in groups and allows establishment of a base line for concentration measurements. The diols are esterified with benzenesulfonyl chloride in pyridine to render them soluble in carbon disulfide prior to infrared measurements. A polarographic method has been developed for the determination of purity in insulin (*169*).

PROTEINS AND AMINO ACIDS

Larsson (*87*) has reported a study of the practicability of determining amino acids and polypeptides by spectroscopy without first hydrolyzing.

VITAMINS

Free vitamin A and esterified vitamin A may be separated by utilizing an alumina column (*35*). The alcohol is eluted with 80–20 petroleum ether–acetone and the Carr-Price color assay utilized to evaluate the sample. Another method for the analysis of vitamin A (*65*) by means of chromatography plus spectroscopy has been reported, which the author believes should supplant the method of Morton and Stubbs for correcting for interfering substances. Another method for the estimation of vitamin A (*93*) in the presence of interfering materials is similar to the Morton-Stubbs correction, except that points selected for direct reading are on either side of the wave length of maximum absorption and are equidistant from it. Still another paper on the determination of vitamin A (*157*) in fish liver oils suggests that the potassium soap micelles formed in saponification absorb vitamin A, making its extraction difficult. The procedure includes addition of barium chloride to precipitate the soap and to break up the micelles. The extraction with chloroform is then only a one-step process. A study having to do with identification and determination of biochemical substances by shifts in the ultraviolet absorption spectra and the application of this work to the determination of ascorbic acid and pyridoxine (*156*) has been reported by Vacher *et al.*

GENERAL

Infrared data covering 79 curves of sugars and their derivatives (*84*) and of some cellulose derivatives have been reported by Kuhn. Spectral range is 2 to 15 microns. A very sensitive colorimetric determination of glucose (*117*) based on the reduction of ferricyanide ions has been described by Park and Johnson. The range of this method is said to be 1 to 9 micrograms of glucose in 1 to 3 ml. of sample. Halogenated methanes (*81*) yield, under certain conditions, when analyzed polarographically wave heights that are said to be proportional to the halides. The solvent is 2 to 1 methanol-water and the supporting electrolyte is 0.1 *M* tetramethylammonium bromide.

Significant trends in pharmaceutical analysis continue to develop as the newer analytical techniques and instruments receive wider and wider application. It is evident that infrared spectrometry is beginning to enjoy quantitative as well as qualitative utilization on a par with the ultraviolet and visible spectrometric techniques. Chromatographic procedures continue to improve as aids in the separation and identification of components of complex mixtures. An appreciable number of reports of the use of anhydrous media in the titration of Lewis acids and bases of pharmaceutical interest also appeared during the year. A great many applications of these and other techniques remain to be explored.

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Natural and Synthetic Rubbers

NORMAN BEKKEDAHL
National Bureau of Standards, Washington, D. C.

THIS is the third of a series of review articles to appear in this journal on analytical methods pertaining to natural and synthetic rubber. Although the first review (*15*) covered only chemical methods, the second (*13*) was broadened to include physical testing. The present review covers both, and refers to articles appearing in the journals for the year ending about October 1950. Like the previous review, it omits test methods applied to compounding ingredients and to materials used in the manufacture of synthetic rubbers. It does not in general refer to procedures that are more concerned with problems of fundamental research than with testing methods. It is also restricted to procedures which have already been applied to the analysis and testing of natural or synthetic rubbers, rather than to general procedures which may find future application in this field.

GENERAL INFORMATION

SURVEYS

Besides the review papers mentioned (*13*, *15*), several other surveys also appeared within the past year. In a series of review articles on chemical engineering materials for construction purposes Fisher (*52*) covered the general field of elastomers and Peters surveyed (*151*) that of hard rubber. Both authors referred chiefly to those properties of the materials which are important for purposes of construction. Kreuter (*103*) presented a review and discussion of the testing methods used in the rubber industry, and included representative recipes for testing rubber vulcanizates. At the Second Rubber Technology Conference held in London, the testing papers of which were reviewed last year (*13*), an open discussion was held on the standardization of methods for measuring tensile strength, hardness, abrasion, and tear resistance of vulcanized rubber (*41*). In the latest "Annual Report of the Progress of Rubber Technology" Drakeley (*45*) wrote the chapter which covers physical and chemical testing of rubbers and latices. The Fifth Foundation Lecture of the Institution of Rubber Industry was presented on May 10, 1950, in London by van Rossem (*163–165*). In his lecture he discussed the properties of natural rubber as a raw material, including its variability, and the need for standardization of methods for evaluating its qualities.

In an exhibition of scientific instruments and equipment the Institut Français du Caoutchouc (*110*) displayed the developments of its technical department for the first time. Included were the rebound meter, a viscometer equipped with spheres, a modulometer, an abrasion tester, a consistometer, and a stabilometer. In a "photo story" the Firestone Tire and Rubber Co. (*171*) described its developments in the field of testing, which included a ring-modulus machine for evaluating modulus characteristics of cured rubber samples, a densimeter for measuring the specific gravity of materials which are plastic at room temperature, a hand-held penetrometer for measuring hardness of cured rubber compounds, a flexometer for measuring heat generation and fatigue in rubber and rubber-fabric composition during flexing, and a plastometer which evaluates the plastic flow characteristics of unvulcanized stocks by extrusion.

BOOKS

Barron (*8*) published the third edition of his "Modern Synthetic Rubbers." Maffei and collaborators (*120*) prepared a book in Portuguese on the technology of rubber, which was based on a series of lectures given under the auspices of the São Paulo Rubber Manufacturers' Association and the Institute of Technological Research. It is excellent as a text for purposes of student training, and contains chapters on the physical and chemical testing of rubbers and on specifications for rubber goods which are based on American and British standards. Houwink (*77*) published Volume I, General Theory, of the series on "Elastomers and Plastomers." *Rubber Age* (*177*) in late 1949 came out with another revised edition of its popular "Rubber Red Book."

Dawson and Thomsett (*42*) prepared in mimeographed form an "Annotated Comprehensive List of Trade Names of Synthetics," which contains an alphabetical listing of substitutes and extenders for natural rubber, synthetic rubbers, synthetic resins, and plastics. This comprehensive collection of over 8000 entries should ease considerably the troublesome problems of dealing with trade names and also with commercial and technological terms. Although LeBras and Delalande's new book in French, "The Chemical Derivatives of Natural Rubber" (*107*), is not so closely connected with the testing of natural and synthetic rubbers, it deserves mention here as the only book of its kind to describe natural rubber derivatives comprehensively.

STANDARDIZATION

A.S.T.M. Committee D-11 on Rubber and Rubberlike Materials issued its latest book on standards (*3*), which includes over 100 methods covering all types of testing on rubber products. A report of the committee (*4*) lists the various proposed changes in the standards together with some new additions. The

British Standards Institution (*29*) revised its 10-year-old bulletin of standards, B.S.-903, composed of methods for testing vulcanized rubber. The new issue has been greatly enlarged to include synthetic as well as natural rubber. Each method or group of closely related methods is now in effect a self-contained document, and is accordingly given a distinctive number appended to the main number 903. The German Verein Deutscher Ingenieure (V.D.I.) (*209*) has issued directions for the formation and application of rubber parts, and refers to the D.I.N. and V.D.E. specifications covering rubber goods.

Definite progress seems to have been made at the September 1949 meeting of the International Organization for Standardization, Technical Committee 45 on Rubber (*31*, *79*, *155*, *185*), which was held at The Hague. Buist (*31*), Powell (*155*), and Scott (*185*) give considerable detail as to the agreements obtained at The Hague in tests on tear, hardness, abrasion, adhesion, and aging. The accomplishments of a later meeting of the committee held in Akron, Ohio, during October 1950, have also been enumerated (*176*). At this meeting the delegates discussed hardness, tension, tear strength, ply adhesion, aging, abrasion, and flex cracking, as well as the testing of rubber latex, and the new French system of grading raw rubber.

CHEMICAL ANALYSIS

The old Weber test for the chemical identification of natural rubber has been redescribed in a somewhat modified form by Stern (*199*) because of its increased importance since the introduction of synthetic rubbers. Nitsche and Toeldte (*146*) identify and characterize substances of high molecular weight from tests on solubility behavior and precipitability in different groups of solvents under definite conditions. The rates of solution in standard and auxiliary solvents are noted, as well as the type of precipitate, and whether or not the latter redissolves in an excess of solvent. Kress (*102*) and Koch (*97*), by means of selective absorption of ultraviolet radiation, were both able to distinguish between a number of vulcanization accelerators in master batch stocks. Déribéré (*43*) subjected various rubber coloring agents to infrared and ultraviolet light, and found some interesting methods for their identification.

Narayanan (*133*, *134*) describes the procedures used for the more common quantitative chemical tests on rubber and latex, and also includes some of the less common tests, such as loss of weight of crude rubber on washing.

The British Standards Institution describes the quantitative estimation of free sulfur in rubber by the copper spiral method (*29*), in which a strip of copper gauze is placed with the acetone in the extraction apparatus; the free sulfur is allowed to combine with the copper to form a sulfide, which is then analyzed for sulfur content. Mann (*123*) made an examination of the response of the copper spiral method to pure sulfur-containing accelerators and to extracts from vulcanizates. He concluded that the presence of certain accelerators may lead to error, but that this error is usually less than that obtained with other methods.

When using the Grote combustion micromethod for the determination of sulfur, Walter (*212*) prefers a different method for determining the end point of the titration of the sulfate by means of standard barium chloride. Instead of using the usual internal visual indicator, he determines the end point by means of a photometric method. Reznik (*160*) describes a method for the microdetermination of sulfur in vulcanized rubber by fusion with potassium in a sealed capillary. The potassium sulfide is oxidized with excess iodate and back-titrated with thiosulfate. Galloway and Foxton (*56*) describe two methods for determining the presence of elemental sulfur in minute quantities, such as in "blooms," because these methods are not well known in the rubber industry. In the first method the bloom is treated with sodium hydroxide and pyridine. The presence of sulfur gives a transient blue-green coloration, which is rapidly succeeded by an orange-brown color. In the second procedure the bloom is ground with Schönberg's reagent, benzylimidodi-(*p*-methoxyphenyl)-methane, heated to 210°; and treated with a few drops of benzene. A blue coloration indicates the presence of sulfur. On addition of a mercuric chloride crystal the color slowly fades, while the crystal assumes a red or orange tint.

Poulton and Tunnicliffe (*154*) describe a method for the determination of copper in rubber, which is much simpler than those usually recommended by the standardization organizations. They recommend a careful dry-ashing procedure for the removal of organic material in place of the wet-oxidation method because of the saving of time. The influence of iron can be eliminated by measuring the optical density of the carbamate complex in solution of carbon tetrachloride before and after treatment with potassium cyanide, which completely removes the brown color due to copper alone. If the iron concentration is not high, the simplified and more rapid procedure is suggested, in which the copper diethyl dithiocarbamate complex is formed from the sodium compound in an aqueous medium.

Naughton and Frodyma (*138*) describe a method and apparatus for the microdetermination of carbon and hydrogen in organic compounds which can be completed in 16 minutes. The sample is burned in a modified Pregl apparatus, and the water vapor and the carbon dioxide are collected in a dry ice trap and a liquid air trap, respectively. After the excess oxygen is pumped out, these gases are measured manometrically. The accuracy obtained is claimed to be very good.

A method for the determination of free carbon in cured rubber stocks is described by Kolthoff and Gutmacher (*101*); the sample is softened in boiling *p*-dichlorobenzene before treatment with *tert*-butylhydroperoxide in the presence of osmium tetroxide. No difficulties are encountered in filtering the carbon. No correction factor is used. The method has been applied successfully to GR-S, Butyl rubber, and neoprene.

Hale, Hale, and Jones (*65*) recommend that for determining the nitrogen content of materials such as natural rubber, where the nitrogen content is low, and the conventional Kjeldahl method therefore not convenient, a semimicro digestion technique be used, and the nitrogen content be determined colorimetrically.

Harris, Smith, and Mitchell (*68*) have modified the Unterzaucher method for the direct quantitative determination of oxygen in organic materials. The oxygen is converted into carbon monoxide, which is determined quantitatively by means of a thermal conductivity bridge, giving continuous recordings of potential during the course of the pyrolysis and conversion reactions.

Dowden (*44*) finds that a potentiometric determination of zinc oxide in rubber is a much simpler procedure than that which requires an outside indicator to find the end point of the titration. During the potassium ferrocyanide titration a pH meter is used, with platinum and constantan wires as electrodes. Poulton and Tarrant (*153*) use a polarographic method for determining the quantity of zinc in compounded rubber. In their method the operations are conducted in an acid medium in order to eliminate inaccuracies due to coprecipitation of zinc by phosphates originally in the rubber. The new method is applicable over the complete range within which zinc is likely to occur in rubber.

Tryon (*206*) made improvements in the apparatus for the determination of small quantities of moisture in rubber by the azeotropic method of distillation, using toluene. His improvements not only increase the precision of the method but also greatly speed up the procedure and make it more convenient for operation.

Maffei and Outa (*121*) developed a new method for determining quantitatively the amount of rubber in latex. Potassium bromate, potassium bromide, and hydrochloric acid are added to

the aqueous dispersion, and the excess bromine is titrated with potassium iodide and sodium thiosulfate. The results agree very well with the coagulation method. This procedure can also be extended to the analysis of solid rubber by dissolving it in carbon tetrachloride and then forming an aqueous dispersion of it with the aid of bentonite.

Lee, Kolthoff, and Johnson (*108*) describe a method for determining the unsaturation of Butyl rubbers and certain branched olefins by the use of iodine monochloride. The usual high results obtained by similar earlier methods, caused by steric strain and decomposition brought about by the addition of the reagent, have been corrected by taking into account the slower addition reaction for the decomposition products.

Schaefer (*180*) noted visual changes which take place during chemical reactions involving some of the more common accelerators for rubber vulcanization. The accelerator is dissolved in acetone, copper sulfate solution is added, and the appearance is then observed by color reaction, crystallization, or precipitation which will distinguish and identify the accelerator present. It was found possible to identify most of the accelerators either in the pure state or in a vulcanizate.

Kendall and Phillips (*89*) developed a method for determining the thickness of wax bloom on the surface of vulcanized rubber. Twists of defatted cotton or wool moistened with light petroleum are wiped over a known area of surface. The wax bloom is then extracted from the twists and weighed.

AGING

The oxidation of rubber has been the subject of a review article by van Amerongen (*5*). Esch (*50*) reviewed and discussed aging tests, and gave reasons for the selection of 70° C. as the most used temperature for conducting these experiments.

For the determination of resistance of rubber to sun checking the R. T. Vanderbilt Co. (*208*) recommends the use of tapered-strip test pieces. When these trapezoid strips are held in a stretched position in a frame it is possible to get an infinite number of different elongations on them between zero and that desired. Samples exposed to the sun are observed for the distribution, density, and depth of cracking. The extension corresponding to maximum cracking can be read off by means of a template.

Nellen, Dunlap, Glaser, and Landes (*140*) studied the effect of atmospheric ozone on tires during storage, and found it to depend on various conditions, such as geographical location, temperature, type and construction of storehouse, and ventilation. Their tests were conducted at a number of different locations in the United States, in which they found the atmosphere to vary considerably as to ozone concentration.

An ozone aging tester, which is claimed to generate and maintain a continuous flow of ozone under controlled conditions of temperature and pressure, has been developed by the G. F. Bush Associates (*83*), Princeton, N. J. It is called the GFB-LGL ozonator. A new weatherometer, called Type XW, has been introduced by the Atlas Electric Devices Co. (*173*). It is a completely redesigned and modernized version of the older model and incorporates many new features.

Blum, Shelton, and Winn (*25*) studied the results of aging tests on rubber samples of different thicknesses. Their purpose was to establish a safe limit of thickness of sample, so that the chemical reaction and not the diffusion is the limiting factor. They concluded that the conventional 0.075- to 0.080-inch thickness is frequently too great, especially for the later stages of oxidation, which are autocatalytic. Shelton and Cox (*193*) investigated a volumetric oxygen-absorption test for aging natural rubber and compared it with the conventional air-oven and oxygen-bomb tests. The effect of cure was found to be very slight in the case of the oxygen absorption test, somewhat greater in the oxygen-bomb test, and very pronounced in the air-oven method.

Mesrobian and Tobolsky (*128*) believe that other chemicals besides oxygen affect the chemical reactions involved in the aging of rubber. Conventional aging tests do not always correlate well with practical performance, and supplementary tests may be required, such as the determination of stress relaxation by a modified apparatus and technique described in their paper. Lawrence and Shelton (*106*) describe applications and their techniques used in measuring oxygen absorption, and note various stages of oxygen addition for compounds structurally related to GR-S. Kuźminskiĭ and co-workers (*104*) found that when a rubber is subjected to fatigue under an alternating stress of constant frequency (250 cycles per minute and amplitude of 50%) in addition to the oxygen treatment the oxidation proceeds at a much more rapid rate.

Scott (*186*) studied the effects of oxygen and temperature on the aging of GR-S vulcanizates. He concluded, as did Mesrobian and Tobolsky (*128*), that in addition to promoting loss of tensile strength, oxygen can be the cause of both stiffening and softening. The former effect is obtained from cross linking, and the latter from chain scission. Scott (*186, 187*) found that above 80° C. increased oxygen favors softening. Oxygen-bomb tests at 70° C. and air-oven aging at 100° C. therefore may not be suitable for simulating natural aging conditions for GR-S vulcanizates. Undercures were found to be particularly susceptible to softening.

Vacca, Erickson, and Lundberg (*207*) made aging tests on black neoprene jackets used for outdoor telephone wire. They found that there was little change in tensile strength with time, but that the elongation decreased fairly rapidly. This trend was true not only for the outdoor aging but also for air-oven aging at 70° to 150° C. This loss of elongation, however, was not necessarily indicative of short-life service.

Kirchhof (*93*) analyzed various types of reclaim for hardness, acetone-solubles, chloroform-solubles, ash, rubber-hydrocarbon content, and behavior on air-aging at 100° C. He concluded that the property which gave him the best indication of the true value of a reclaim is its aging at 100° C.

Newton and Wake (*143*) describe an apparatus and special technique in which they measure the increase in bending modulus of thin sheets of rubber exposed to air and light. They studied the effect of different wave lengths of light on light stiffening and surface crazing, and compared their tests with the conventional scratch and folding tests of proofing. They concluded that the modulus test was much more sensitive and would detect changes long before the other methods. They were able to measure the stiffening of the rubber samples within one week. Soden and Wake (*196*) studied the deterioration of rubber under the influence of dry and moist heat in addition to being subjected to light. Rubber articles and proofings were exposed to tropical atmospheric conditions and the results of these aging tests were compared with those obtained from aging in ovens under both moist and dry conditions. The results of the tests did not place the rubbers in the same order. It is therefore necessary to expose the samples to suitable light radiations as a part of any accelerated "tropical" test.

Villain (*210*) studied the action of copper and its derivatives on the aging of rubber. In his tests he used the Geer oven at 70° C., the oxygen bomb at 20 kg. per sq. cm., oxygen absorption, and natural aging (8 months), using tensile strength as the criterion. He concluded that although most copper compounds are pyro-oxygenic agents and accelerate the aging, copper may also appear in nondeleterious forms. The same copper compound may, of course, have a different effect on different rubbers, depending on the chemical composition of the compound.

LATEX

Van Gils (*62*) reviewed the common tests performed on latex and stressed the necessity for universal standardization in the procedures. The newest annual issue of the A.S.T.M. standards

(*1*) contains for the first time procedures for tests and specifications for concentrated creamed or centrifuged latices. For the test on mechanical stability of the latex an apparatus is specified which makes use of a high-speed stirring technique. In the United States such an apparatus has been designed by the Firestone Tire and Rubber Co. and is being produced by the Precision Scientific Co. (*174*). In France a similar apparatus was designed by the Institut Français du Caoutchouc (*126*) and is known as the I.F.C. stabilometer.

The use of the potassium hydroxide number as the sole specification for the chemical stability of latex is strongly objected to by van Gils (*63*). He says that there is no correlation between the potassium hydroxide number and zinc oxide stability test, and therefore advocates the use of both tests to get the whole picture. Hayes (*71*) studied the stability of compounded latex by thickening experiments with zinc oxide, and showed that the maximum thickening effect takes place when the zinc oxide concentration is less than 1% of the dry rubber content of the latex. Gelation tests with sodium silicofluoride indicated that the curve of the pH *vs.* zinc oxide concentration is closely related to that of the viscosity *vs.* zinc oxide concentration, and he proposes a theory to account for the maximum thickening effect. By successive creaming operations with incremental additions of ammonium alginate Schmidt and Kelsey (*183*) were able to separate Hevea latex into separate fractions, and thus determined a correlation between particle size and alginate concentration. Researchers at the Indonesian Rubber Institute (*139*) have been able to separate Hevea latex into white and yellow portions in stable liquid states, each having different properties.

McMullen (*118*) developed an apparatus and technique by which it is possible to extract latex from the tree under completely aseptic conditions and in the absence of oxygen. He was able to manipulate a tapping knife inside a steel body so as to penetrate the latex vessels of the tree under vacuum-tight conditions. The preparation of latex under sterile conditions such as this should enable one to study the mechanism of natural coagulation and also the deterioration of latex under ordinary conditions.

Bächle (*6*) studied the drying speeds of latex films and confirmed earlier conclusions that the temperatures should not be too high because of formation of bubbles in the film, that drying takes place in two phases, and that drying is slower in the presence of moisture. He also found that filler content has a decided effect on the rate of drying. Blevins, Wright, and Leonard (*22*) improved the casting of elastomeric test films from latices, and obtained flaw-free films by casting against a surface of gypsum so as to obtain drying from both sides of the film.

McGavack and Bevilacqua (*117*) studied the absorption of oxygen by ammonia-preserved latex and showed that the addition of less than 0.2% of oxygen on the total solids of the latex lowers the Mooney viscosity of the resulting rubber from 110 to 40 units, with indications of still lower viscosities with further additions of oxygen. They conclude, therefore, that it is extremely important that the user or seller of latex should clearly understand to what extent his latex has been exposed to oxygen.

UNVULCANIZED RUBBER

MOLECULAR PROPERTIES

The characteristics of macromolecules and their size, with many references to previous articles on the subject, have been the subject of a paper by dePonte (*152*).

Clement (*38*) describes an apparatus and osmometric technique for the determination of high molecular weights and polymolecularity of high polymers. The design of his apparatus is such that the osmotic pressure exerted on the semipermeable membrane presses it against a grill, thus eliminating the effect of ballooning.

The fractionation of natural rubber by means of organic solvents has been the subject of review and discussion by Belmas (*16*), in which he points out that the knowledge of the nature of sol and gel fractions has become more precise and that the molecular weights vary from 9000 to 300,000. Gavoret and Magat (*57*, *58*) developed a rapid method for the determination of molecular weights of high polymers to an accuracy of within 10% by means of measuring the precipitation threshold. With alcohols as a precipitant it was possible to determine the molecular weights of narrow fractions. Léger and Giguère (*109*) found that the molecular weight distribution curve for GR-S by the coacervate method was very similar to that obtained by fractional precipitation. They observed a sharp peak in the curves at a value of about 40,000. Cragg and Simkins (*40*), by intrinsic viscosity measurements on five fractions of GR-S, determined the effects of chain length and various solvents on the viscosity-temperature coefficient. They found the coefficient to be positive for poorer solvents and slightly negative for the better ones.

Benedict, Brooks, and Puckett (*17*) fractionated resin-free rubber from guayule plants and found it to vary in molecular weight, depending on the part of the plant from which it was obtained. As guayule rubber contains from 1 to 2% of rubber hydrocarbon with molecular weight of from 2000 to 20,000, which is soluble in acetone, Meeks, Banigan, and Planck (*127*) report that the usual method of determination of resins by acetone extraction gives false results when applied to guayule rubber.

Rochow and Rochow (*162*) examined the molecules of silicone rubber by means of the electron microscope technique at 10,000 to 50,000 diameter magnifications, and found their molecular weights to be of the order of about 300,000. The values obtained by this method agree with those by osmometry.

Gehman (*60*) emphasizes the value of infrared absorption spectra and x-ray diffraction for determining the molecular structure such as cis and trans configurations and also crystallization. By infrared analysis and also by methods of separation by solution, Schlesinger and Leeper (*181*, *182*) found a single chicle plant which produced a product consisting of a mixture of caoutchouc (cis) and gutta (trans) forms of polyisoprene. This observation disproves the popular belief that a single plant species produces only either the cis or the trans form of polyisoprene and not mixtures of the two.

Koch (*97*) applied spectrographic methods to studies in vulcanization. He was able to follow the chemical transformation of accelerators to their products in rubber compounds, to identify sulfur linkages in cross-linked polymers, and to prove the participation of a free-radical mechanism in the vulcanization process. Hauser and le Beau (*69*) used ultramicroscopy by incident light to study the morphological changes which occur during vulcanization.

Madorsky (*119*) pyrolyzed samples of various synthetic rubbers at temperatures from 350° to 450° C. and at a pressure of 10^{-6} mm. of mercury, and separated the products into two solid fractions, two liquid fractions, and a gaseous fraction. In this way he was able to study the mechanism of chain rupture.

Campbell and Allen (*36*) studied the crystallinity of high polymers by direct observation with the polarizing microscope. Their results check reasonably well with those obtained in dilatometric studies, and are believed by the authors to be more sensitive than those obtained from measurements of x-ray diffraction. Wiley, Brauer, and Bennett (*215*) continued their researches on the refractometric method for the determination of second-order transition temperatures in high polymers, and by applying good insulation to their Abbé refractometer they extended the lower range of temperatures to −120° C. Ichimura (*80*) theorizes on the cause of the second-order transition of natural rubber as evidenced by specific heat measurements, while Kikuchi presents his theories on the crystallization (*90*) and melting (*91*) of rubber. Both authors used data of other observers published several years previously. Mayo (*125*) shows that crystallization can cause changes in results obtained from tests on physical properties

such as stress-strain, hardness, and compression set. Measurements obtained by means of x-ray diffraction are correlated with the results of the other tests. Fox, Flory, and Marshall (*55*), in their studies on the thermodynamics of crystallization in high polymers, found that the critical elongation for incipient crystallization in stretched vulcanized rubber, as determined by density measurements, is dependent on the temperature. Crystallization was found to set in at an elongation well below that at which the stress-strain curve assumes a steep slope.

The thermodynamics of rubberlike elasticity has been the subject of discussions by several authors. Ishihara (*86*) presents a statistical theory on the network structure of rubberlike elasticity. Katz (*88*) gives a thermodynamic analysis of one-component and two-component elastic systems, and states that the basic hypothesis of the kinetic theory of rubber elasticity leads to conclusions which are thermodynamically impossible. Bartenev (*9*) gives relationships between thermodynamic properties for the ideal rubber, and states that the analogous equations of Wiegand, Snyder, James, and Guth, and others are inexact. Lode (*112, 113*) believes that the theories as developed by Meyer, Kuhn, Wall, and Treloar do not explain all the phenomena of rubberlike elasticity and therefore takes a new approach to the subject. Thirion (*202*) states that there is a close relationship between the structure of rubbers and mechanical properties, and believes that in the future research methods involving instruments such as x-rays, electron diffraction, and electron microscope will play a more important role.

Scott and Magat studied the thermodynamics of solutions of high polymers. Solvents and polymers were first classified according to "density of internal energy," which permits a prediction of their solution properties (*122*). When the polymers are cross-linked, however, the solubility is zero, and a study was made of the swelling characteristics of the vulcanizates in different solvents (*189*).

VISCOSITY

According to Penn (*150*), most of the variability of rubbers is caused by the variations in their molecular weights. This author believes that the Mooney viscosity value can be taken as an indication of the molecular weight. Eccher (*47*) describes a cylindrical rheometer of the Couette type which is suitable for the experimental determination of the relationship between the rate of shear and the shear-stress of rubber and rubber compounds.

Blow and Schofield (*23*) point out both the advantages and the disadvantages of the shearing-disk viscometer, and make measurements on the stress-relaxation of unvulcanized rubber by means of the Mooney instrument. They report that the recovery of GR-S and natural rubbers as measured by the Mooney viscometer correlates fairly well with recovery measurements made on a parallel-plate plastometer and also obtained from shrinkage measurements on the rubbers after milling, calendering, and extruding. Conant, Hall, and Lyons (*39*) studied the equivalent effects of time and temperature in the shear-creep and recovery of elastomers, and set forth an explicit relation for the time-temperature dependence of the viscoelastic phenomena in polymers.

Kilbourne, Misner, and Fairchild (*92*) studied the plasticity of various types of reclaimed rubbers as measured by various methods, such as Williams' parallel-plate, Mooney shearing-disk, Firestone extruder, laboratory mixing, and factory processing in Banbury. They concluded that the Mooney instrument gives the most precise results. Milling actually measures some property other than plasticity. No single test can replace both milling and Mooney measurements, and when these two tests are used in conjunction with one another the maximum amount of information is obtainable as to the processibility of reclaimed rubber.

In the latest standards of the American Society for Testing Materials the use of the shearing-disk viscometer is described in determining curing characteristics and in evaluating scorch properties (*2*). Somerville and Maassen (*197*) studied scorch characteristics as determined by the Mooney at various temperatures, and recommend the use of a higher temperature, 250° F., as very near the ideal for most compounds. Measurements made below this temperature are too time-consuming, and those made above this temperature cause the reaction to proceed too rapidly for proper detection of the scorch.

Glikman, Vladykina, and Perepelova (*66*) claim that methods involving capillary flow and rotation are unsuitable for the determination of apparent viscosity of elastic high-polymer solutions, and recommend a method using falling spheres for the characterization of structural viscosity and thixotropy. Kolbanovskaya and Rebinder (*98, 99*) measured the viscoelastic properties of rubber solutions by determining time dependence of the shearing suspended by a twisted vertical torsion wire in an outer cylinder containing the rubber solution.

A rotational viscometer manufactured by the Precision Scientific Co. (*37*), Chicago, Ill., produces consistency curves for viscosity instead of single point measurements. It consists of a rotating sample cup and a stationary bob immersed in it. The viscous drag on the bob is imposed on a coil spring which is twisted through an angle measured on a calibrated disk. Willenberg and Fritz (*218*) designed a new viscometer which is adaptable to very heavy liquids. It consists of two cylinders connected by a capillary and two loaded pistons, the liquid being forced either way through the capillary. Bestul and Belcher (*18*) studied the flow behavior of concentrated GR-S rubber solutions, and give an equation for representing the flow curves which involves actual shear rate at viscometer wall, shearing strain, and two constants.

MISCELLANEOUS

Van Rossem and Hoekstra (*74, 166*) studied the mastication of natural rubber by several different types of plasticity measurements. Plasticized rubber was found to harden slowly. Storing rubber at elevated temperatures causes hardening for the first few days, and then a slow softening. Exaggerated mastication of rubber decreases the tensile properties of a vulcanizate made from it. No correlation was found, however, between the viscosity of the rubber before plastication and the modulus after vulcanization. Blow and Wood (*24*) studied the mastication and compounding of natural rubber in an oxygen-free atmosphere, and confirmed Cotton's and Busse's experiments, which showed that very little breakdown of rubber takes place in the absence of oxygen.

Wood and Fanning (*219*) found that extraction of the resins from guayule rubber can be accomplished more efficiently if mastication of the rubber takes place during the extraction. For this apparatus they made use of an adapted laboratory internal mixer connected to a continuous extraction apparatus.

The problem of preventing the fingers of a mill-roll operator from being trapped in the nip of a horizontal two-roll mill became of interest to Lunn (*114*), who made a mathematical and theoretical study on the safe-working areas of mills. He concludes that the safety area increases as roll diameter increases, but that the absolute safe area is restricted. It can be defined, provided that the stopping power of the brake is known.

A new pyrometer for the measurement of surface temperatures is provided by the Pyrometer Instrument Co., Bergenfield, N. J. (*172*).

EVALUATION AND GRADING

It has long been the view of rubber technologists that natural rubber should, in addition to its present system of visual grading, be evaluated according to its intrinsic properties. Recent articles (*64, 115, 116, 163–165, 190, 198*) indicate that a technical system of classification is even more necessary today because of the in-

creased competition between the natural and synthetic rubbers. First to develop and introduce a new system of grading were the Syndicat des Planteurs de Caoutchouc d'Indochine and the Union des Planteurs de Caoutchouc (*201*), who began marking the bales of natural rubber produced in Indochina according to three classes of plasticity and three classes of the rate of cure (*157*, *158*, *178*). This new system does not interfere with the usual system of grading by appearance only. The French system of classification of rubber met with considerable interest and soon was recommended for trial by rubber growers from all parts of the Far East (*64*, *78*, *116*, *159*, *165*, *178*, *184*). At the recent meeting of the International Organization for Standardization, Technical Committee 45 on Rubber, held in Akron, a resolution was adopted (*176*) by the delegates that the French technical system of grading be applied to the usual market grades of natural rubber.

An interest in the technical classification of natural rubber according to intrinsic properties was also expressed by the consumers of raw rubber, and the American Society for Testing Materials introduced a new Subcommittee on Crude Natural Rubber in its Committee D-11 on Rubber and Rubberlike Materials (*4*). This new subcommittee has already had three meetings (*14*, *82*, *85*, *175*), and is concentrating its present activities on the quantitative determination of dirt in rubber and the development of test recipes and methods for evaluating the physical properties of natural rubber vulcanizates (*82*).

Newton, Philpott, Smith, and Wren (*142*) studied the variability of Malayan crude rubber as to Mooney viscosity and vulcanization characteristics. They found that the visual grades of ribbed smoked sheet Nos. 1, 2, 3, and 4 differed only slightly in the properties examined, and could see no technical justification for downgrading smoked sheet on account of the presence of bubbles. Rubber quality variations were large from estate to estate by the system of technical grading but not by visual inspection.

Fletcher (*53*) stated that tests on abrasion, flex-cracking, cut growth, etc., are important properties for a rubber but are difficult to measure. He believes that the National Bureau of Standards strain tester (*75*) shows a high degree of merit, but prefers and describes a simplified version of this instrument for use chiefly on plantations and other out-of-the-way places. He prefers to conduct tension testing at low elongations where there is no crystallization in the rubber, and recommends measuring the minimum strain and also the time of vulcanization required to attain this minimum value. Blackwell, Blow, Fletcher, Mullins, and Wood (*20*) continued work for the British Rubber Producers' Research Association on the variability of natural rubber. They directed their attention, however, to the processing characteristics of the rubber, which are important prior to the vulcanization. Uniformity of processing characteristics is necessary in order to ensure constant extruder and calender performances and to maintain constant modulus of the vulcanizates. Blackwell, Blow, and Fletcher (*19*) studied vulcanizate stiffness for raw rubbers of different characteristics, different mixing formulas, and different mixing procedures. They believe that the minimum strain of any rubber at a fixed compounded viscosity can be calculated from the minimum strain at any other known compound viscosity.

Gee (*59*) makes note of the wide differences of opinion as to the meaning of the "rate of cure." He discusses the arguments for and against each of the three major methods—viz., time to reach maximum modulus, scorch time, and hyperbola parameters. Although most of the investigators are of the opinion that a single cure is all that is required to determine a rate of cure, others (*46*, *85*) believe that at least three times of cure are necessary. Dunlap, Glaser, and Nellen (*46*) used an inclined plane tester to determine the intrinsic low-stress properties of rubber compounds. By making a hysteresis loop test at low stresses over a series of cures, an extremely accurate measure is provided for determining the rate and state of cure. They believe that the results from their tests for hysteresis loss made at low elongations come closer than those made at high elongations to simulating conditions obtained in actual use in many commercial products such as tires.

VULCANIZATES

STATIC TENSION AND COMPRESSION

The N.B.S. strain tester (*75*), which measures elongation of rubberlike materials of virtually motionless specimens at a definite period of time after the application of a predetermined load, is being manufactured by the G. F. Bush Associates, Hopewell, N. J. (*168*). The British Rubber Producers' Research Association has adopted the use of a strain tester for tension measurements because it gives more precise results than can be obtained from modulus testers. The association (*28*) designed a simplified version of the N.B.S. strain tester which it believes will be suitable for the grading of natural rubber and can be more conveniently adapted for use on plantations. It uses molded test pieces, similar to those used for T-50 measurements, and gives values of strain up to 250% elongation.

The Société Fenwick and the Institut Français du Caoutchouc (*195*) designed an apparatus, which they call the I.F.C. modulometer, for the express purpose of determining modulus at 600% elongation. It can use either dumbbell or ring specimens. A new and improved universal testing machine, of 60,000 pounds' capacity, called Baldwin Model 60-H, is now in production by the Baldwin Locomotive Works, Philadelphia, Pa. (*169*). It can be loaded either in tension or compression by hydraulic means.

Villars (*211*) designed a high-speed stress-strain machine which is capable of recording stress-strain values for an elastomer at elongations up to 270% per millisecond. By means of this instrument it is possible, by examining the stress-strain curves, to determine whether or not a rubber crystallizes upon stretching.

Baxton and Vodden (*10*) developed an unbonded resistance strain gage which they find useful in an apparatus used for measuring static stresses in rubber which take place for several hours or days. They found that the usual type of gages cause a drift in the experimental values because of plastic flow of the adhesive between the gage and its supports.

Thum and Derenbach (*204*) discussed the various factors which influence the tensile strength of natural and synthetic rubbers. They found that for short-time tensile measurements the results differed from 10 to 20% between samples stretched parallel and those stretched perpendicular to the direction of milling.

Newton (*141*) pointed out that a fallacious method of comparing experimental results was used in an article by Klute (*95*) on the effect of die surface irregularities on the results of tensile tests for vulcanized rubber. Klute (*96*) took cognizance of Newton's correction and recalculated his data. In general, however, the original conclusions of his work were not altered by the different results.

Beatty and Juve (*12*) describe an apparatus and technique for measuring stress relaxation of rubber vulcanizates in compression, and discuss the effect of temperature, degree of deformation, and compounding on the relaxation of stress. Their method gives results with reasonable speed and accuracy. Wilkinson and Gehman (*217*) also devised an apparatus for making similar measurements. They make use of the expansion of a Sylphon bellows by air pressure to compress small cylindrical test pieces. As stress in the sample decreases owing to relaxation, air is automatically released through a valve so as to maintain the force at a magnitude just sufficient to keep the sample at a constant compression strain. Tohara (*205*) made measurements in an apparatus of different design, which makes use of a lever, supported on one side by ball bearings and attracted on the other by an electromagnet, to apply constant compression instantaneously. The stress is measured by piezoelectric crystals.

Moakes and Pyne (*129*) studied the properties of silicone rubbers and came to the conclusion that compression set was the most valuable and sensitive property for assessing the rate of vulcanization of the silicone rubbers.

HARDNESS

The measurement of hardness has been the subject of a paper by Buist (*30*), who describes the four principal methods used in industry, British Standards Institution, American Society for Testing Materials, DVM, and Shore, as using large contact pressure for hard stocks and low contact pressure for soft stocks. He developed the Imperial Chemical Industries tester, which uses constant indentation. The measurements obtained with the new instrument were found to be linearly related to those obtained from compression modulus.

Scott (*188*) reports that the precision of hardness measurements by indentation under load may be increased by eliminating or decreasing the friction error of the instrument. This can be accomplished by the application of gentle vibration, such as with an electrically operated buzzer. Lubrication of the surface of the test piece by means of talc also gives further improvement.

ABRASION

Buist (*32*) states that abrasion resistance is not a specific property of a rubber, but is dependent on the technique of measurement. He classifies twenty-one abrasive machines into four categories. Nearly all machines were developed for a special compound, but service life is usually forecast very poorly from these laboratory tests. It is possible, however, to obtain improvement in results if an extraction of the test pieces is first made in ethyl alcohol–toluene azeotrope.

The Goodyear angle abrader, which was designed by the Goodyear Tire and Rubber Co. primarily for testing tire tread compounds, is now being manufactured by Scott Testers, Inc., Providence, R. I. (*170*). The machine accommodates eight samples at a time, which are placed against the flat face of a vitreous alundum grinding wheel that rotates on a vertical shaft at 80 r.p.m.

An abrasion machine, the improved Model 140 abrader, has been developed by the Taber Instrument Corp., North Tonawanda, N. Y. (*84*). The new model incorporates a number of improvements in design but uses the same principles as the older model.

ADHESION

Borroff and Wake (*27*) continued their work on the adhesion between rubber and textiles. Their most recent work concludes that the strength of a rubber-to-fabric bond is a simple function of the number of fibers ruptured in breaking the adhesive bond and of the strength of the individual fibers.

Buist and Naunton (*34, 35*) criticize the A.S.T.M. method of testing adhesive bonds as giving values for the total unit strength and not the bond strength alone. They describe a new and better method of evaluation in which the bonded unit is tested by impact of a falling weight. Kaercher and Blum (*87*) describe test methods for the evaluation of comparative bonding strengths of Butyl rubber to brass. The tensile pull in pounds per square inch is measured normal to the plane of bonding.

Van Rossem and Vercruijsse (*167*) modified an older apparatus and test method for measuring the tackiness of masticated and compounded rubber mixes. The new apparatus allows for the application of an ordinary dynamometer, and the separating force is measured. Its chief use has been the determination of adhesive strength of rubber flooring with various cements.

TEAR

Buist and Geldof (*33*) made an extensive comparison of the crescent and the Delft methods for measuring tear strength, in which they used vulcanizates of different types made from natural and various synthetic rubbers. Both methods placed the vulcanizates in the same order. Interlaboratory variations were somewhat greater with the Delft method, and they show that both methods are superior to the angle method. The authors believe that the property measured should be called tear strength, and the results should be expressed in load per unit area. Graves (*67*), on the other hand, thinks the results should be in load per unit thickness of specimen, because the load is not distributed uniformly over the area. He also criticizes other statements made in Buist's papers, and says that the angle test specimen shows excellent discrimination between different rubber samples. These authors seem to have divergent interpretations in the evaluation of tear resistance in elastomers.

DYNAMIC TESTS

The June 1950 issue of the *Transactions of the Institution of the Rubber Industry* is devoted entirely to papers and discussions on dynamic properties of rubber which were presented at a symposium on the use of rubber, as an engineering material. Waring's contribution (*214*) was concerned with dynamic testing in compression, in which he made comparisons between the ICI electrical compression vibrator and the I.G. mechanical vibrator (Roelig machine). In general, the correlation of hysteresis data between the two was rather poor. Waring prefers the ICI vibrator for measurements involving small amplitudes, but for larger amplitudes, such as are found in tire service, the I.G. instrument gives better results. Mullins (*132*) designed an apparatus to demonstrate and overcome the shortcomings of the forced-vibration technique. The test specimens are subjected to repeated cycles of constant alternating sinusoidal deflection, and the transmitted force is continuously recorded. This gives a complete stress-strain hysteresis loop for steady-state vibrations. Fletcher and Gent (*54*) report data on dynamic modulus and viscosity of bonded rubber test specimens in shear. Attention is called to the practical importance of the results of this apparatus and to dynamic properties in general for the use of rubber as an engineering material.

Hillier (*72, 73*) gives a brief description of a method for measuring the propagation and attenuation of longitudinal oscillations in filaments of rubberlike materials. Results obtained from measurements made on this apparatus were compared with those made on an ordinary tension machine, and it was found that directly comparable values were obtained for the dynamic and static moduli. Rivlin (*161*) discusses the equation conventionally employed for evaluating the elastic and viscous coefficients in dynamic experiments on rubber where these coefficients depend on the frequency and amplitude of vibration. A justification of its use for steady state experiments in the range of linear behavior was presented.

Nielsen (*144*) describes and illustrates two types of dynamic apparatus in which stresses vary sinusoidally at a rate from subaudio up to the audio range of frequencies. The first is a torsion pendulum, from which can be obtained the shear modulus and a mechanical damping term. The second is a resonance reed vibrator, data from which can be used to calculate a Young's modulus of elasticity and mechanical damping for rigid films. Nolle (*147*) gives data for the complex dynamic Young's modulus for natural and various synthetic rubbers in the temperature range $-60°$ to $+100°$ C. and frequency range 0.1 to 10^5 cycles per second. His results reveal a strong similarity between viscoelastic behavior of rubberlike materials and the dielectric behavior of polar compounds.

Marvin, Fitzgerald, and Ferry (*124*) developed and described an apparatus for measuring the dynamic viscosity and rigidity of soft rubberlike solids in small oscillating deformations. Their apparatus has two advantages over the more familiar resonance devices. The amplitudes of motion, which need not be measured directly, are extremely small and will therefore

minimize any nonlinear effects or temperature changes from heat dissipation, and a continuous range of frequencies spaced as closely as desired is available without adjusting the masses. Hopkins (*76*) describes an apparatus for the determination of dynamic properties of elastomers in shear at audio frequencies, and typical values are given for shear modulus and viscosity for several elastomers, including natural rubber, Butyl, and silicone.

Natta and Baccaredda (*135–137*) give experimental data on the ultrasonic velocity and density for natural and some synthetic rubbers and for some of their more common solvents. These velocities obtained experimentally are compared with those calculated on the basis of bond velocity and on the radicals which make up the molecules. The ratio of the experimental to the calculated values gives a so-called form factor, which can be used to decide the question of branching and to determine the positions of polymerization additions such as 1,2; 2,3; or 1,4.

Nielsen, Buchdahl, and Claver (*145*) describe a recording torsion pendulum of a new design for the determination of dynamic properties. Baldwin (*7*) found it possible to adapt the Yerzley oscillograph to measure dynamic modulus and internal viscosity of synthetic rubber compounds, and thus determine the effects of compounding ingredients, polymer structure, and other factors. Kolsky (*100*) investigated the mechanical properties of materials at very high rates of loading. He describes a method of determining the stress-strain relation of materials when the stresses are applied in the order of 20 microseconds. The results obtained under these extremely rapid conditions of loading are very different from those where the forces are applied more slowly.

Enabnit and Gehman (*48*) describe a technique for measuring dynamic properties which can be used for both raw and vulcanized polymers. Their experiments show that despite variations in curing rate among polymers there is a definite correlation between the dynamic properties of a series of raw polymers and those of vulcanized gum and tread stocks prepared from them. It therefore appears possible with suitable background to anticipate fairly well from measurements on the raw polymers the dynamic properties which can be achieved in a vulcanized compound. Waring (*213*) studied dynamic properties as applied to problems of reinforcement. He found it possible to obtain reproducible results in dynamic modulus of tread stocks and to estimate the general level of reinforcements if sufficient attention is paid to the control of factors such as static load, bonding of the test sample, temperature, temperature history, amplitude of vibration, and vibration history.

In order to study hysteresis of elastomers at high deformations and at fairly high amplitudes, Mooney and Black (*130*) developed what they termed a spider hysterometer, which measures energy loss per cycle directly and continuously during operation. Wilkinson and Gehman (*216*) modified the original Roelig machine, particularly with respect to photographing instead of paper-tracing the hysteresis loops. They also studied operational variables of the machine, such as duration of test, frequency, and static and dynamic load, and give data on heat generation, dynamic modulus, dynamic resilience, and internal friction. Thirion and Martin (*203*) made a systematic study on the hysteresis of rubber under low elongations (0 to 100%) and low speeds. Because of the low degree of accuracy of the present lever dynamometers, they designed a piezoelectric quartz dynamometer, and took pictures on the screen of a cathode ray oscillograph of the extension and retraction curves of the load at different elongations of rings and dumbbell specimens.

Labbe (*105*) studied the rebound characteristics of polymer compounds at various temperatures as low as −75° C. by means of the Goodyear-Healy rebound pendulum apparatus and by the Bashore resiliometer. The Bashore instrument, with air as coolant, is to be preferred over the Goodyear-Healy apparatus, chiefly because of its efficiency and economy of operation. Boonstra (*26*) measured impact resilience as a function of temperature by means of the Lüpke pendulum. He used a special accessory electrical apparatus to measure the time of contact at impact, which was of the order of 3 to 4 milliseconds.

A method has been developed by Story (*200*) whereby separate indexes may be obtained for the flex life and heat build-up properties of elastomers. These indexes were found to be independent of the state of cure but dependent on the test recipe and the dispersion of the compounding ingredients. The method may be used to examine the effect of polymerization variables, compounding ingredients and procedures, or ambient temperature, on these two properties.

LOW TEMPERATURE TESTS

A review was given by Liska (*111*) of low temperature characteristics of rubber elastomers and the methods for testing these properties. Shaw (*192*) reviewed many papers and described many test methods and instruments used in the evaluation of low-temperature properties of rubbers. He classified all methods of test into only a few categories.

Gehman, Jones, Wilkinson, and Woodford (*61*) followed the progressive stiffening of elastomers by measuring the relative torsional modulus of test strips held up to 2 months at temperatures ranging from −20° to −60° C. They reported that the general stiffening of the elastomers may be supplemented by the occurrence of crystallization if the elastomers have sufficient regularity of molecular structure and other conditions are favorable. The percentage of combined sulfur, of course, has a controlling influence on the spontaneous stiffening, especially with natural rubber.

Smith, Hermonat, Haxo, and Meyer (*194*) describe a retraction test employing large deformations, based on the well-known T-50 test, which can measure the merits of a rubber for low-temperature applications. Data obtained from this test show a correlation between its results and those of cold-compression set and hardness after storage.

Morris, Hollister, and Barrett (*131*) discuss the significance of cold-compression set of vulcanizates as regards internal viscosity, second-order transition, and tendency to crystallize. They show experimentally that the ability of a gasket to maintain a seal when compressed for extended periods of time at low temperatures can be foretold from compression-set tests. Boonstra (*26*) states that impact resilience measurements show minimum values at temperatures closely related to brittle points.

A brittle-point tester for elastomeric materials has been designed by the American Cyanamid Co. and Scott Testers, Inc. (*81*), in conformity with the A.S.T.M. specifications. It is an extremely compact apparatus and operates on a solenoid-activated guillotine principle with a prescribed standard test piece.

MISCELLANEOUS

Saunders (*179*) measured the birefringence of vulcanizates elongated under known load, using Nicol prisms and a Babinet compensator. He produced his vulcanizates by peroxide reaction, used no sulfur, and removed all by-products from the vulcanizate. He found that the relation between stress and birefringence was linear and reversible up to about 100% strain.

Odynets (*149*) used an optical method for measuring the dielectric constant and dielectrics such as ebonite in the centimeter wave-length range.

Oberto (*148*) examined rubber vulcanizates under transmitted polarized light to find optical evidence of the presence of anisotropy caused by movements of particles during calendering, extruding, or molding operations. Using microtome sections of the rubber a microscope can be used at low magnifications. A transparent gum compound can be used to study particle orientation, but more details are noticed if a small quantity (1%) of carbon black is present.

Blake, Kitchin, and Pratt (*21*) studied the failures of rubber insulation caused by soil microorganisms, and described the method

of microbiological attack on natural and synthetic rubber insulation. Some rubber vulcanizates made from rubbers which are not attacked by microorganisms may still be subject to electrical failures because of attack by microbes on other materials present in these compounds.

Setchkin (*191*) describes an apparatus and method for determining the ignition characteristics, such as the flash temperature and the self-ignition temperature, for plastics and rubbers.

Haworth and Pryer (*70, 156*) studied staining problems associated with rubber chemicals by exposing them to ultraviolet light and sunlight, and classified them as coloration, discoloration, staining by migration, and finger marking.

Beatty and Cornell (*11*) developed tests for both laboratory and full-scale equipment to measure the important properties connected with rubber bearings. These tests are used to measure the coefficient of friction and the wear.

Endres, Coleman, Pierson, and Sinclair (*49*) studied the effect of synthetic elastomers on the properties of petroleum asphalts and their possible use in road surfaces. The important properties measured were increase in softening point, decrease in penetration, and improved low-temperature characteristics.

Fainshtein (*51*) devised a method for the determination of heat resistance of ebonite, in which a load is applied at the center of a thin sample of the hard rubber supported by its periphery, and the temperature is noted at which the center sags.

Kirchhof (*94*) developed a simple and precise method for determining the so-called relative firmness in different factices. A small sample of each factice is ground and placed in a test tube and tapped to settle to constant volume, the volume is noted, the sample is heated to 150° for 30 minutes, and then the contraction and other distinguishing characteristics are noted.

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WATER ANALYSIS

S. K. LOVE, *U. S. Geological Survey, Washington, D. C.*

THIS is the third annual review of analytical procedures applied to the analysis of water and reported in the technical literature. The first review (*53*) covered a 5-year period ended in the fall of 1948 and the second review (*54*) covered the year 1948–49. This review covers papers published during the past year, plus a few papers published in earlier years which were not previously included.

Increasing use of instruments in the analysis of water is apparent. Photometers and spectrophotometers of various kinds have largely displaced visual observations in colorimetric analysis. Better instruments, making possible greater precision, are being placed on the market. Flame photometry is finding wider application, and, under carefully controlled conditions, good results are being reported. The new type of balance, which weighs at constant load, is gaining favor where speed as well as accuracy is important.

Several of the analytical procedures to which references are made relate to the analysis of water-borne industrial wastes. With the increasing emphasis on the control and abatement of pollution of natural waters, there is a growing need for methods of analysis for determining constituents frequently present in wastes but not ordinarily found in unpolluted waters. Widespread interest in this field will undoubtedly result in the publication of an increasing number of papers on the analysis of industrial waste materials.

CALCIUM AND MAGNESIUM

It appears that separate values for calcium and magnesium can be obtained by means of the new direct titration method for hardness. By use of a suitable indicator, usually ammonium purpurate, calcium can be determined directly with an accuracy ordinarily equal to that obtained by the oxalate-permanganate method in the average laboratory. The direct calcium method was reported favorably by Marcy (*58*). Magnesium is calculated by difference using the calcium and the total hardness titration volumes. Walker and Robertson (*94*) determined

magnesium by precipitating calcium as the oxalate and titrating the remaining hardness due to magnesium with a salt of ethylenediaminetetraacetic acid.

HARDNESS

In the review for 1949 it was pointed out that a new method for the direct titration of hardness was rapidly gaining favor, but that very little information about the method had been published. During 1950 there was a landslide movement toward the adoption of the direct method for determining hardness, which was accompanied by the appearance of a considerable number of papers in the literature. The method depends on the ability of the sodium salt of ethylenediaminetetraacetic acid to sequester calcium and magnesium ions quantitatively.

The outstanding features of the direct colorimetric titration method are speed, precision, accuracy, and simplicity. The soap method at its best falls far short of the new method in all of these characteristics. The colorimetric titration is made by adding a standard solution of the tetraacetate salt to a measured volume of sample, usually in a 50- or 100-ml. aliquot, in the presence of a suitable dye, ordinarily eriochrome black T. The color change representing the end point is sharp, distinct, and reproducible.

Among the papers first describing the new method in detail were those by Betz and Noll (*10*), Diehl, Goetz, and Hach (*23*), and Diehl (*22*). Certain ions interfere with the titration, especially iron, copper, and manganese. Directions are given for removal of the interfering ions or for use of special reagents to eliminate interference. Other papers by Marcy (*57*, *58*), Betz and Noll (*11*), McCrumb (*56*), Rossum and Villarruz (*73*), and Willey and Senger (*99*) report favorable laboratory experience with the method. A study by Goetz, Loomis, and Diehl (*37*) indicates that the standard tetraacetate solutions are stable to within 1% over a period of 4 months.

Janssen and Spruitt (*47*) reported further refinements in the Clark (soap) procedure by plotting the time that lather remains unbroken against the volume of soap solution used. The 5-minute period is found by interpolation. The authors stated that sodium oleate is preferred to sodium stearate.

COPPER, IRON, ZINC, AND LEAD

The dithizone method for determining copper was applied by Swope, Hattman, and Pellkofer (*85*) with appropriate modification for sewage and industrial wastes. Values are read on a spectro- or filter photometer at 510 mμ. Accuracy of 0.231 p.p.m. of copper in 16 replicas of sewage with standard deviation of 0.0175 p.p.m. was reported. In another paper, Swope, Jaffe, and O'Callaghan (*87*) reported on the determination of metals in industrial wastes using the *o*-phenanthroline method for iron and diethyldithiocarbamate (electrolytic) method for copper.

Gad and Manthey (*32*) reviewed the thiocyanate method for total iron in water and gave a procedure for a rapid thiocyanate–nitric acid–permanganate method. The presence of tannates, humic acids, and other organic compounds in natural waters has frequently led to difficulty in the determination of iron. There has been considerable evidence that iron is found in organic molecules which do not yield the iron quantitatively in the ordinary procedures of analysis. Bastisse (*8*) reported new evidence of complex organic iron molecules in irrigation drainage waters.

Zinc has been determined polarographically by De Salas and Graells (*21*). A sensitivity of 0.5 p.p.m. is reported.

The dithizone method for lead was studied by Buczkowska (*14*) using tartaric or citric acid to prevent precipitation of other metals. No interference was observed up to 10 p.p.m. of iron and copper, 10 p.p.m. of zinc, and 20 p.p.m. of manganese. As little as 0.5 p.p.m. of tin produced up to 35% error. The method is sensitive to 0.01 p.p.m. of lead.

SODIUM AND POTASSIUM

Although the flame photometer appears to have gained wide favor in the determination of sodium and potassium, very little has been published on this application during the past year. West, Folse, and Montgomery (*97*) described the Beckman flame photometer and the use of radiation buffers in the determination of sodium and potassium. The instrument was also recommended for calcium. Connors (*19*) mentioned the instrument in a brief paper discussing advances in methods of water analysis.

Mestayer (*59*) determined sodium by precipitating it as the complex salt $3UO_2(OAc)_2.Mg(OAc)_2.NaOAc.8H_2O$. After centrifuging, the precipitate is dissolved in acetic acid, and color is developed with potassium ferrocyanide. Concentrations of lithium and strontium over 0.5 p.p.m. interfere, but potassium up to 4 grams per liter can be tolerated. Potassium was determined by precipitating as cobaltinitrite, dissolving in concentrated sulfuric acid, and titrating with potassium permanganate. Cesium, rubidium, and ammonium over 0.2 p.p.m. interfere.

pH, ALKALINITY, AND CARBON DIOXIDE

Additional studies have been made on the calculation of the various forms of alkalinity from the pH and total alkalinity. Fähnrich (*27*) presented nomographs showing the interrelationships and discussed errors made by authors of earlier papers in arriving at corrections for high concentrations of dissolved solids. Papp (*65*) took exception to formulas published by Tillmans, Kolthoff, Langelier, and others for calculating the pH of soft waters which are in a "lime–carbon dioxide balance." He stated that the difference between actual and calculated pH values increases as the hardness decreases. The errors are said to be due to failure to take into account the calcium bicarbonate in solution. Papp developed formulas for calculating pH for waters of varying degrees of hardness. In another paper Papp (*64*) calculated the pH of various concentrations of calcium bicarbonate solutions free from carbonic acid. He found that $pH = 7.076 + \log 2k/2$ where k is the chemically bound carbon dioxide content of the solution in milligrams per liter.

The determination of pH of industrial waste water is often affected by the nature of the wastes. For leather-tanning wastes Evlanova (*26*) recommended the glass electrode or colorimetry using isoamyl alcohol for extraction of the original color. For textile plant wastes the glass electrode was preferred, except that the hydrogen electrode was used for bleaching wastes. For sulfite paper plant wastes the glass electrode or colorimetry was recommended.

CHLORIDE

The time-honored silver nitrate (Mohr) procedure for the titration of chloride in the presence of potassium chromate indicator is probably the most widely used and most generally satisfactory method for ordinary chloride concentrations in water. For concentrations lower than 10 p.p.m., however, most analysts evaporate from 100 to 500 ml. to a small volume in order to obtain accurate results. A method has been developed by Clarke (*18*) which eliminates concentration of a large sample for waters containing less than 10 p.p.m. It is a modification of the method of Dubsky and Trtilek (*24*), in which chloride is titrated with mercuric nitrate in the presence of diphenylcarbazone indicator. Clarke presented both titrimetric and colorimetric procedures. An accuracy of ±0.5 p.p.m. for concentrations up to 200 p.p.m. chloride is reported.

The chlorinity (chloride, bromide, and iodide) of sea water was determined by Buljan (*15*) by the usual silver nitrate method, but using different indicators including potassium chromate, sodium arsenate, and fluorescein. Fluorescein gave accurate results, provided coagulation of silver chloride was prevented. This can be accomplished by keeping the chloride concentration

between 0.066 and 0.053 N, or by adding 2 ml. of 1% gelatin to 15 ml. of sea water diluted with 30 ml. of distilled water. Potassium chromate gave some error and sodium arsenate even more.

The use of protective colloids in the argentometric determination of chloride ion was reported by Stalzer (*82*). Gum arabic, gelatin, dextrin, and agar agar were used with varying results.

FLUORIDE

According to Ballczo (*7*), fluoride is readily determined by removal from water by steam distillation with perchloric acid and titration of the distillate with thorium nitrate to form a lake with alizarin. Results are reported to be "excellent" down to 0.1 p.p.m. but subject to increasing error at lower concentrations.

A direct method was reported by Thrun (*93*) using an aluminum lake of eriochromecyanine to produce a color change from red to pink to orange. For high fluoride concentrations distillation is recommended prior to color development.

A modification of the ferric thiocyanate procedure developed by Foster (*28*) was described by Ingols *et al.* (*45*). The pH of the sample is adjusted to 1.9 to 2.0 with perchloric acid. After addition of ammonium thiocyanate, ferric alum, and zirconium oxychloride, the color of the sample is compared with a blank containing equal amounts of reagents. Correction is made for sulfate, which interferes.

A photometric study of interferences in the alizarin method for fluoride was made by Taras, Cisco, and Garnell (*89*). They recommended the use of sodium thiosulfate or ultraviolet irradiation to correct for interference due to free chlorine, and hydrogen peroxide to reduce interference due to manganese.

NITRITE AND NITRATE

The pink color formed by nitrite with resorcinol is the basis of a method described by Sánchez (*76*, *77*). Chlorides, sulfates, carbonates, and small amounts of nitrates do not interfere. Nitrates can be determined by the same method by heating the sample with sulfuric and hydrochloric acids. Baillie (*5*) has given directions for the preparation of a standard curve for determining nitrite with a spectrophotometer at 525 mμ.

A photometric study of the phenoldisulfonic acid method for nitrates was made by Taras (*88*). He confirmed the generally acknowledged need for removing chloride if present in more than very small amounts. Although there is some indication of loss of nitrate when the evaporated residue is treated with phenoldisulfonic acid, treating the sample with sulfuric acid prior to evaporation to eliminate the bicarbonate results in greater losses. Taras concluded that samples should be evaporated without adjusting the natural alkalinity.

A study of the brucine method for nitrate was made by Gad, Knetsch, and Schlichting (*31*). They concluded that best results are obtained when nitrate (as N_2O_5) is in the range of 4 to 10 mg. per liter. Alekin and Chernovskaya (*2*) examined the Noll method for nitrates to determine best conditions. The importance of size and nitrate content of the sample and the need for uniform reaction time were stressed.

A micromethod for nitrates was reported by Leithe (*51*) using ferroin indicator and titrating with potassium dichromate after boiling with ferrous sulfate, sulfuric acid, sodium chloride, and potassium bicarbonate.

PHOSPHATE

Sulfamic acid was added to molybdate reagent by Greenberg, Weinberger, and Sawyer (*38*) to control interference of nitrite up to 25 p.p.m. in the colorimetric determination of phosphate. The amount of hexametaphosphate remaining in water after threshold treatment was determined colorimetrically by Young and Golledge (*101*) by the molybdate reaction. Tannins, when present, were removed with decolorizing charcoal. Excess phosphate in boiler water was determined by Petatskiĭ (*67*) by using cation exchange resins.

CHLORINE

Methods for the determination of free chlorine in water were reviewed by Gad and Schlichting (*35*). In discussing the interference of iron and manganese in the dimethyl-*p*-phenylenediamine and the *o*-tolidine colorimetric methods, they pointed out that (*a*) iron can be fixed with basic sodium phosphate, and (*b*) after the chlorine is converted to chloramine the manganese can be removed by the addition of calcium carbonate. Gad and Priegnitz (*34*) also described a procedure for determining chlorine by use of methyl orange, methyl yellow, or methyl red. Methyl orange appears to give best results. Iron is reported not to interfere.

A sensitive colorimetric procedure for determining free chlorine (or bromine) was reported by Milton (*61*). It involves the use of sodium cyanide and pyridine containing a small amount of benzidene hydrochloride. The reaction produces intensely colored dianil derivatives.

A patent was issued to Wallace (*95*) for a device which will record high concentrations, or produce audible or visual signals when predetermined concentrations of chlorine in water are exceeded. Methods for determining the chlorine hydrolysis products in water were reported by Hermanowicz and Dozanska (*41*).

DISSOLVED OXYGEN

Although the Winkler method is generally recognized as the best method for determining dissolved oxygen, many useful modifications are constantly being worked out. For determining low concentrations of dissolved oxygen in degassed water, Delassus, Devaux, and Montigny (*20*) have reviewed existing methods and have reported favorably on two methods for oxygen in the range of 0.000 to 0.072 mg. per liter. In the upper part of this range the Perley potentiometric method is preferred. In the lower part of the range a colorimetric method utilizing the yellow color developed by *o*-tolidine with chlorine liberated from the Winkler reagent is said to give reliable results. An accuracy of 2% for concentrations below 0.003 mg. per liter is reported.

Another modification of the Winkler method for determining dissolved oxygen in deaerated water for concentrations between 0.005 and 0.02 ml. per liter was described by Arnott, McPheat, and Ling (*4*). The liberated iodine is extracted with carbon tetrachloride. Sodium thiosulfate is added in excess and back-titrated with standard iodine solution. Maximum error of 0.002 ml. per liter of oxygen is reported.

Dissatisfaction with the Rideal-Stewart modification of the Winkler method prompted Cameron (*16*) to devise a procedure whereby there are added to a 4-ounce bottle of sample the following: 0.5 ml. of a 40% solution of manganese sulfate, and 0.5 ml. of a solution containing 33% sodium hydroxide, 20% potassium iodide, and 0.8% sodium azide. After 10 minutes, 100 ml. are titrated with 0.0125 N sodium thiosulfate.

A titrimetric method for determining dissolved oxygen without the use of iodine-containing reagents was described by Gad (*29*). It is a modification of the Leithe procedure using manganese chloride, sodium hydroxide, sodium pyrophosphate, and diphenylamine in sulfuric acid. The final titration is made with 0.01 N ferrous sulfate.

The use of various preservatives including xylene, chloroform, and mercuric chloride was studied by Alekin and Voronkov (*3*) to prevent loss of dissolved oxygen in samples stored under different conditions. Mercuric chloride was found most effective. Storage in an alkaline condition and at the temperature at which samples are collected is recommended.

BIOCHEMICAL OXYGEN DEMAND AND OXYGEN CONSUMED

The B.O.D. test continues to be the subject of intensive study. Although it is not a precision test and is subject to numerous errors and misinterpretations, it still appears to be the best single means of determining the oxygen-consuming potential of water. One of the difficulties in B.O.D. work is agreement in the use of primary standards. Glucose and glutamic acid have been recommended by Sawyer *et al.* (*78*) as suitable standards.

A review of the fundamental background of biological oxidation of industrial water was published by Heukelekian (*42*). Attention was given to food, seeding organisms, and environmental factors. Ingols (*44*) reported on a study of the variation of B.O.D. with time, using the rate of decolorizing of methylene blue as a possible measure of the reaction. He concluded that the rate of decolorizing could not be correlated with the B.O.D. of the sample.

Experiences with modifications of the B.O.D. test were reported by Mohlman *et al.* (*62*). Efforts were made to suppress nitrification. Pasteurization and reseeding, as well as acidification and neutralization, did not appear to depress the normal B.O.D. of raw sewage or Imhoff tank effluents, but did reduce the B.O.D. of activated sludge appreciably. Chromium salts reduced the B.O.D. of raw and settled sewage to a very great extent. The depressing effect of chromate ions was also reported by Placak, Ruchhoft, and Snapp (*68*). Copper has a similar effect, but not so pronounced an effect as chromium.

The reseeding of B.O.D. bottles at the end of 24 and 48 hours in the absence of oxygen was reported by Borden and Woodcock (*12*) to eliminate interference by toxic materials. In control tests the reseeding raised the B.O.D. of treated waste to values approximating those obtained before treatment. The B.O.D.'s of several common organic compounds were reported by Strong, Shrewsbury, and Hatfield (*84*). Technique for the B.O.D. determinations at 50% oxygen depletion was described by Rogers (*71*). Results of a 6-month study of the Winkler volumetric procedure were published by Lewin (*52*).

The merits of using biochemical oxygen demand or oxygen consumed to measure the strength of sewage and trade wastes were discussed by Ingols, Hildebrand, and Ridenour (*46*). The results obtained from oxygen consumed determinations using the acid-dichromate method were considered preferable. Graphs for determining the proper dilution in the B.O.D. test were presented by Wolfe (*100*), and graphs for determining B.O.D. curve constants were described by Thomas (*91*).

A review of the permanganate procedure for determining oxygen consumed with particular reference to inaccuracies was given by Alciaturi (*1*). Meyer (*60*) compared values obtained with Pleissner's conversion factors and by Fair's method and pointed out a lack of agreement with actual observations. The use of silver as a catalyst in the dichromate reflux method was suggested by Muers (*63*).

MISCELLANEOUS

Hilfiger (*43*) described a method for determining dissolved solids in water in which the sample is passed through ion exchange resin and the liberated acid, equivalent to the adsorbed cations, is neutralized with a standard base. The dissolved solids concentration is calculated as sodium chloride. Metallic silver in water was determined by Gad and Naumann (*33*) by depositing the silver electrolytically and titrating it with potassium thiocyanate in the presence of ferric alum.

A quantitative colorimetric method for boron was reported by Hatcher and Wilcox (*40*). It is based on the reaction of boron and carmine in concentrated sulfuric acid. It is applicable for all concentrations of boron found in water. The ordinary constituents of water, including nitrites and nitrates, do not interfere.

Although scandium is not considered an ordinary constituent in water, Beck (*9*) described a method involving the formation of cubic crystals having the composition $[Co(NH_3)_6].ScF_6$. Color was developed with quinalizarin in isobutyl alcohol. Infrared spectrophotometric determination of deuterium oxide in water was reported by Thornton and Condon (*92*).

An approximate method for the determination of methane was described by Rossum, Villarruz, and Wade (*74*), based on the equilibrium established between methane in solution and the partial pressure of methane in the vapor above the solution.

In order to determine organic carbon in water, Skopintsev (*81*) modified the Krogh and Key method, using a combustion tube attached to a Kjeldahl flask.

A spectrochemical procedure for the analysis of brines was reported by Russell (*75*) to give results within 10%. Spectrochemical methods for mineral constituents in water were also described by Jaycox (*48*).

A system of analysis for small quantities of water was outlined by Gad and Knetsch (*30*) using micromethods. Duggan and Metler (*25*) devised a field kit for the approximate chemical analysis of oil field waters. Directions are given for making the kit. The corrosion potential of oil field brines is determined by analysis of water for unstable constituents such as oxygen, carbon dioxide, alkalinity, etc. Watkins (*96*) discussed methods applicable for analysis of brines.

In a discussion of automatic operations in quantitative analysis, Patterson and Mellon (*66*) included several methods used in water analysis, for such determinations as specific conductance and hardness.

A method was described by Lyne and McLachlan (*55*) for determining trichloroethylene in water using pyridine. As little as 1 p.p.m. could be determined with fair accuracy. Methods for determining trinitrotoluene and hexanitrodiphenylamine in water and industrial wastes were described by Seifert (*80*). Riehl and Will (*70*) discussed precautions to be taken in the determination of phenols in water and trade wastes using the Scott modification of the Gibbs-Sanchis method.

Advances in chemical and colorimetric methods in water analysis were described by Connors (*19*). The paper was primarily concerned with the new procedure for hardness and the flame photometer method for sodium and potassium.

A review of methods for determining bromine in brines was presented by Haslam and Moses (*39*). Phenols were selectively extracted from industrial waste waters by Riediger (*69*) by use of mixtures of esters and ethers of the higher aliphatic alcohols. Photocolorimetric iodometry was recommended by Kulenok (*49, 50*) for the determination of chlorine and dissolved oxygen.

The application of electrical conductivity to water analysis was discussed by Rosenthal (*72*) and by Wilcox (*98*). Methods for the determination of chromium, vanadium, and cyanide in industrial wastes were described by Swope, Hattman, and Pellkofer (*86*).

Studies of changes in chemical composition during storage were made by Chernovskaya (*17*) in which he used chloroform, xylene, ether, and mercuric chloride as "preservatives."

A chromatographic microprocedure was used by Ballczo (*6*) for the determination of lithium. Colloidal sulfur in mineral waters was determined by Garcia-Fernandez (*36*) by use of an organic solvent and Cetosol.

A series of procedures for determining sulfur, boron, and fluoride in thermal waters was published by Schulek and Rózsa (*79*). These procedures are rather involved and probably would not lend themselves to ordinary routine work.

The following books will be of interest to water analysts: "Examination of Waters and Water Supplies" by Taylor (*90*); "Tests for Water Used in Steam Generation" (British Standard) (*13*); and "Chemische und physikalisch-chemische Fragen der Wasserversorgung" by Stooff (*83*).

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Wide-Range Instrument for Controlled Potential Electrolysis

R. W. LAMPHERE

Oak Ridge National Laboratory, Oak Ridge, Tenn.

CONTROLLED potential electrolysis is a promising method for analysis, or quantitative separation of elements from solutions. A number of instruments have been designed for this purpose and are considered in an earlier paper (*2*). In addition, an article by Ashley (*1*) gives an excellent survey of the field and literature to date.

Frequently occasions arise where it is desirable to supply a current of several amperes or a potential up to 50 or 100 volts at the anode of an electrolytic cell—for example, the concentration of reducible ion usually encountered in macro work may involve initial currents of a few amperes. In the case of a side reaction such as gas evolution, much current and also a high

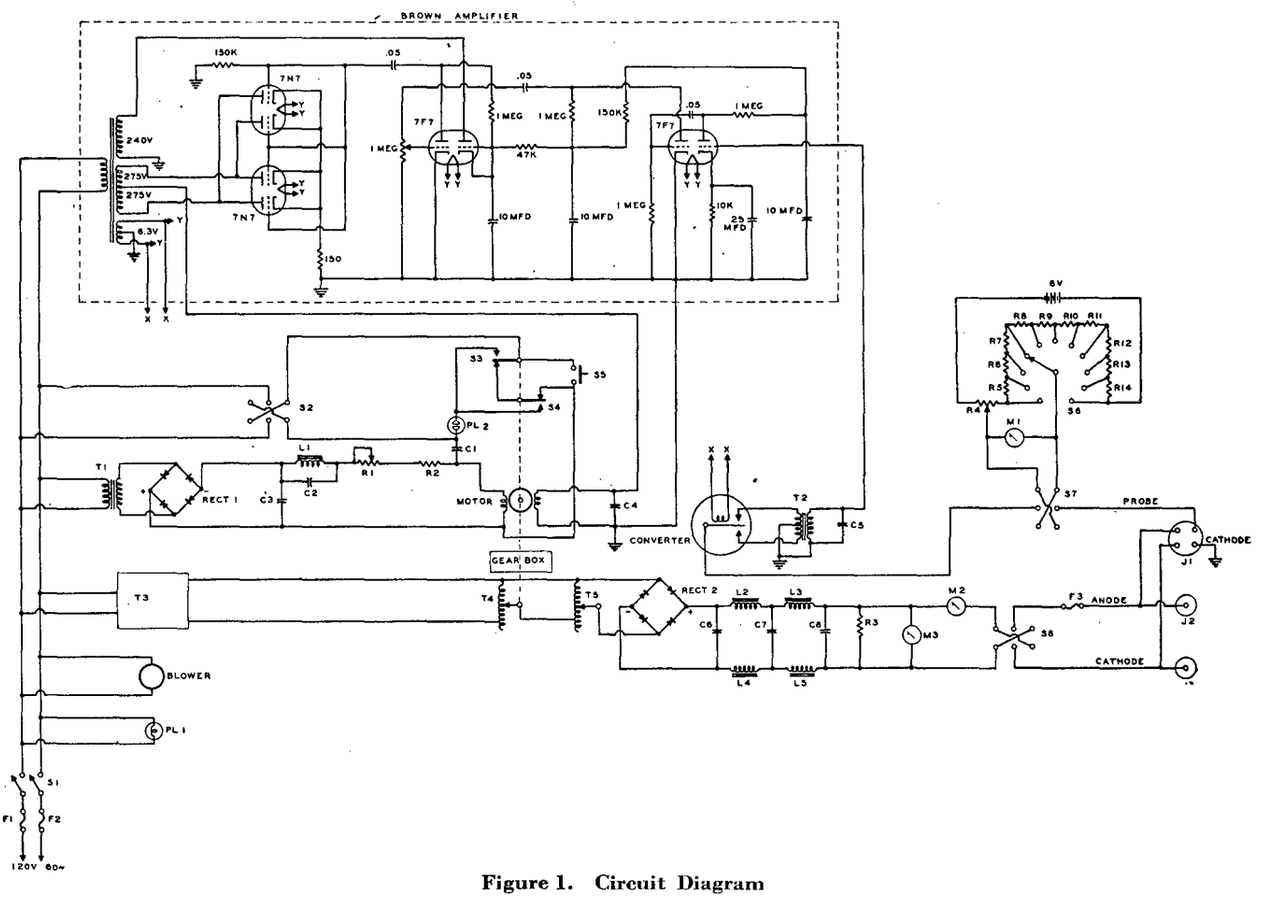

Figure 1. Circuit Diagram

T1. 120 to 120, 40-volt ampere isolating transformer
T2. Unit Transformer Co. 0 to 6 transformer
T3. Sola 120-volt, 500-volt ampere constant voltage transformer
T4. 230-volt 2½-ampere Variac
T5. 115-volt, 5-ampere Variac
L1. 8-henry, 40-ma. choke
L2, *L3*, *L4*, *L5*. 0.16-henry 5-ampere choke
F1, *F2*, *F3*. 10-ampere fuse
S1. Double-pole single-throw 12-ampere switch
S2, *S7*, *S8*. Double-pole double-throw 3-ampere switch
S3, *S4*. Microlimit switch (mounted on *T4*)
S5. Normally open push button, spring return
S6. Isolantite wafer switch, 1 pole 11 positive
C1, *C4*. 1 mfd., 1000 volts
C2. 0.5 mfd., 600 volts
C3. 20 mfd., 250 volts
C5. 0.1 mfd., 500 volts
C6. 500 mfd., 125 volts
C7, *C8*. 1000 mfd., 150 volts
R1. 5000, 25-watt wire-wound potentiometer
R2. 2500, 5 watts
R2. 2500, 5 watts
R3. 3000, 5 watts
R4. 5000, 4-watt wire-wound potentiometer
R5, *R6*, *R7*, *R8*, *R9*, *R10*, *R11*, *R12*, *R13*, *R14*. 2500, ½ watt, 5%
M1. 0 to 50 μa. direct current meter, 1200 ohms internal resistance
M2, *M3*. 0- to 1-μa. direct current meter, 55 ohms internal resistance
Rect. 1. Selenium rectifiers, 75 μa., full wave
Rect. 2. Selenium rectifier, Fansteel V320M
PL1. 120-volt pilot light
PL2. 120-volt neon pilot light
Brown converter
Brown 27-r.p.m. Servo motor
J1. 4-pin chassis connector socket
J2, *J3*. 1-pin chassis connector socket
Gear box, 100/1 reduction
R15. 62.5 ohms, 1% precision
R16. 27.8 ohms, 1% precision
R17. 5.10 ohms, 1% precision
R18. 2.520 ohms, 1% precision
R19. 0.500 ohms, 1% precision
R20. 0.250 ohms, 1% precision
R21. 0.0500 ohms, 1% precision
R22. 195 ohms, 1% precision
S9A, *S9B*. Ceramic wafer switch
S9C. Microswitch normally open

Electrodeposition is a promising method of separating one element at a time from a solution containing several elements. The method is easily carried out by remote control; hence it is particularly suitable for use with radioactive solutions. A closely controlled solution-cathode potential is desirable in order to prevent more than one element from depositing on the cathode. Several amperes of current capacity are useful where concentrations of the desired element are high; also, in some cases, to override the effect of hydrogen evolution. Anode potentials of the order of 50 to 100 volts are sometimes desirable, as in the case of nonaqueous solutions of high internal resistance. In such cases the current requirements are usually low. A simple rugged instrument was built using standard components which will deliver up to 100 volts or 250 volt-amperes, will regulate to 2 mv. over a 12-hour period, and will operate equally well at either polarity. Such an instrument will fulfill practically all requirements for trace separations and will be useful where sizable quantities are to be removed from solution.

anode voltage are required to maintain the solution-cathode potential. For nonaqueous studies the current required may be low but the voltage high owing to the large resistance of the solution. For these reasons an instrument was built which can supply up to 5 amperes, 100 volts, or 250 volt-amperes. The latter limitation simply means the product of current times voltage cannot exceed 250—for example, at 80 volts the current should not exceed 250/80 or 3.12 amperes.

POWER SUPPLY

As shown in Figure 1, power is drawn from the 120-volt, 60-cycle line and goes through the various circuit components in the following order: Sola constant voltage transformer *T3*, regulating Variac *T4*, range control Variac *T5*, selenium full-wave rectifier *Rect. 2*, symmetrical *LC* filter, anode-current meter *M2*, anode voltmeter *M3*, polarity-reversing switch *S8*, anode-current fuse *F3*, and finally to the electrolytic cell.

The Sola transformer prevents ordinary changes in line voltage from affecting the apparatus and also serves to isolate the equipment electrically from the line. In mounting a Sola near sensitive amplifiers it should be borne in mind that it is a strong source of magnetic leakage flux. This radiation is emitted much less strongly in some directions than in others; thus, with a little care it is possible to locate the unit in the same cabinet (Figures 3 and 4) with the control circuit in such a manner that it has no effect on control.

The regulating Variac, *T4*, serves to vary the anode potential (all potentials are referred to cathode as ground or zero) as dictated by the control circuit, thereby holding a constant solution-cathode potential in the cell. It is driven by a Brown motor (part 76750-3, Brown Instrument Co., Philadelphia, Pa.) through a 100 to 1 gear reduction and friction clutch. The clutch permits a quick initial setting to be made by hand at the start of an electrolysis. When seeking balance the Variac turns at a speed that takes 5 minutes to cover its range. This holds the rate of change of anode voltage to 20 volts per minute or less. This rate of change is fast enough for any foreseen plating operation, yet slow enough to permit very close control without hunting. A 240-volt Variac is used to gain the smoother control that results from the greater number of turns (600 turns compared to 300 for a similar 120-volt Variac). The brush has some smoothing effect, presumably because it comes in contact with more than one turn at a time, with the result that the jumps in direct current anode voltage are similar to those from a Variac with 800 turns. If we let ΔV_p equal the step in probe voltage, R the setting of the range control Variac in volts, and V_p the probe voltage in volts, $\Delta V_p = 0.011\ RV_p$ millivolt. R is roughly equal to the anode potential; thus for the worst conceivable condition, $R = 100$ and $V_p = 5$ volts. Then $\Delta V_p = 5.5$ mv. A more usual set of conditions would be $R = 20$, and $V_p = 2$, which gives $\Delta V_p = 0.44$ mv. In each case regulation could not be closer than these limits.

The range control Variac, *T5*, is used to set the upper limit of direct current voltage obtainable from the regulator. Its setting is roughly equal to the direct current anode potential. It is usually advisable to set this only a little higher than the maximum anode potential expected during the electrolysis in order to reduce the voltage per turn on *T4*. This control also is used to vary the direct current output by hand when it is desired to use the machine as a variable voltage power supply. In this case *T4* is turned to its maximum position and the regulating system is shut off.

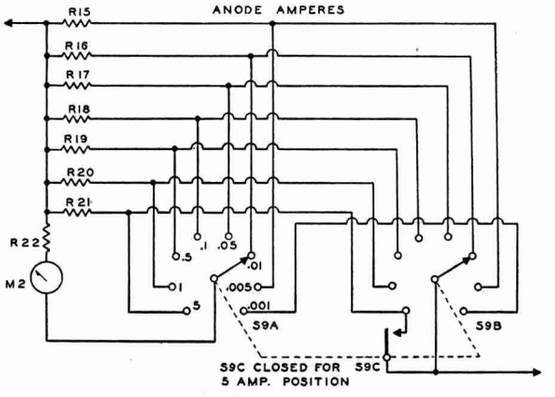

Figure 2. Circuit for Measuring Anode Current

The filter is made symmetrical, since either side of the output may be grounded. Chokes are connected in such a way as to minimize leakage flux (by making mutual inductance of adjacent chokes additive). Otherwise the sensitive balancing circuit might be disturbed. The capacitances are held to the values shown, and a bleeder resistance of 3000 ohms is connected across the output to provide a small enough resistance times capacity constant under all conditions to prevent hunting. (If the resistance times capacity constant is too large the output voltage can only be decreased slowly if cell resistance is high. This causes *T4* to overshoot and results in sustained oscillations.) The reduction factor of the filter is sufficient to limit ripple in anode potential to 2 mv. at 100 volts; hence there is essentially no ripple in solution-cathode potential.

The meters *M2* and *M3* read anode current and voltage, respectively. A range switch with multiplier resistors (not shown) provides ranges of 5, 10, 50, and 100 volts for *M3*. The current ranges are 1, 5, 10, 50, 100, 500, 1000, and 5000 ma.; the circuit used for switching in the appropriate meter shunts is shown in Figure 2. A rather complex arrangement is used which avoids

errors from current times resistance drop in the switch contacts and still permits the use of commonly available components.

REGULATING SYSTEM

The heart of the regulating system is a standard Brown amplifier except for special high impedance input transformer *T*2, and a Brown converter (parts 76020–1 and 75829–1, respectively). Voltage is usually supplied to the probe lead from a reference half-cell and a salt bridge connecting the reference electrode with the solution in the electrolytic cell. This is balanced against a reference voltage set up on potentiometers *R*4 and *S*6. *M*1 gives a coarse voltage reading and has full scale ranges of 0.1, 0.5, 1.0, 5.0, and 10.0 volts. It is common procedure to connect a potentiometer between the reference electrode and cathode in order to read the voltage accurately. If the probe potential differs from the reference voltage, a small current flows through the converter and the input transformer. This current is amplified and causes *T*4 to rotate in a direction that corrects the error. The circuit responds to a ±1-mv. difference, and the probe current is then less than 0.1 μa., a value small enough to prevent polarization of the reference electrode. A separate lead, that carries only the signal current, connects the cathode to the regulator chassis and hence the midpoint of the input transformer.

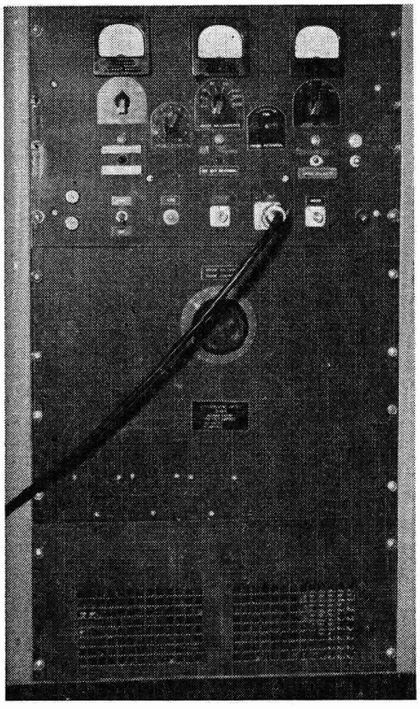

Figure 3. Front View of Instrument

This extra lead to the cathode is clearly necessary to prevent errors which result from current times resistance drop. Additional velocity damping has been added to the Brown motor by feeding direct current from *Rect. 2* into the line phase of the Brown motor. The antiresonant circuit prevents 60-cycle alternating current from feeding back through the rectifier. Ordinarily *R*1 is left at zero, as this gives maximum damping and does not affect regulator sensitivity. To avoid hunting, permissible amplifier gain is limited by response time divided by filter resistance times capacity constant. With the components shown the full gain of the amplifier may be safely used.

OPERATING PROCEDURE

The electrolytic cell is connected to the instrument in the usual way, and the range control is set a little higher than the maximum anode voltage expected during the electrolysis. The machine is turned on and the proper reference potential is established. Subsequent operation is completely automatic. Operating characteristics are the same for either polarity of anode or probe.

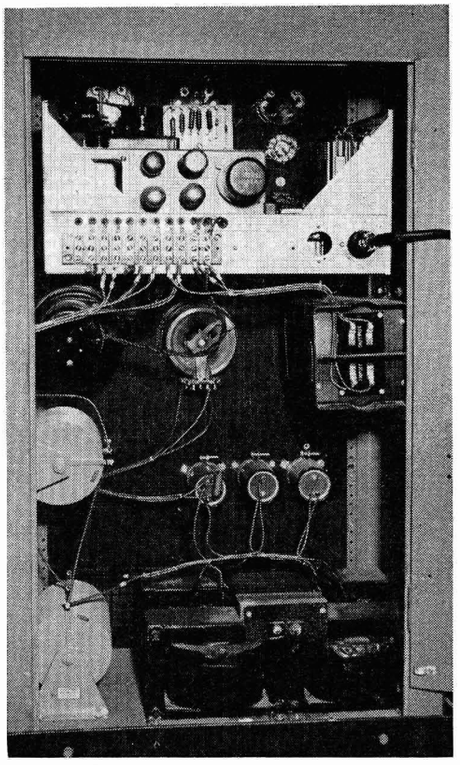

Figure 4. Rear View with Access Door Removed

One such regulator has been in operation at the Oak Ridge National Laboratory for 2 years; others have been in use at Massachusetts Institute of Technology for a shorter period. Experience with these has shown that drift over a 12-hour period is less than 1 mv. Drift is independent of anode current or changes in anode current. Instantaneous departures from reference potential during the course of an electrolysis are within ±5 mv. However, much greater fluctuations may occur where periodic cathode phenomena are encountered. This is discussed in some detail in an earlier paper (*2*). These departures cannot be observed unless a fast response instrument such as a cathode-ray oscilloscope is used to observe probe potential.

The machine has been used as a two-terminal regulator to hold constant anode potential by connecting the probe lead to the movable arm of a variable resistor whose ends are connected to anode and cathode. It can also be used as a constant current source by connecting the probe to a small resistance in series with the cathode lead.

ACKNOWLEDGMENT

The author wishes to thank L. B. Rogers and J. C. Griess for many helpful suggestions and for supplying data obtained during electrolyses. This work was performed at the Oak Ridge National Laboratory under contract 7405 eng. 26, between the Atomic Energy Commission and the Oak Ridge National Laboratory.

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(2) Lamphere, R. W., and Rogers, L. B., *Ibid.*, **22**, 463 (1950).

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Qualitative Analysis from Mass Spectra

S. M. ROCK

Consolidated Engineering Corp., Pasadena, Calif.

Chemical analysis from mass spectrometric data may be either qualitative or quantitative. The latter has been extensively covered in the literature, but the methods used in the former have not been systematically presented. This paper presents several aids to qualitative analysis and outlines and illustrates various basic procedures: check of mass intervals to identify fragments and elements; use of peak-free regions to establish absence of fragments; comparison of peaks with those predicted from normal stable-isotope ratios; recognition of characteristic half-mass and metastable-transition groups; and use of mass spectral compilations such as those available from A.P.I. Project 44. A table of isotope contributions of hydrocarbons through C_7 is given. Peaks are roughly classified by magnitude and charted against mass in a summary of the spectra of 279 compounds. One of the most valuable features of the mass spectrum of a mixture is that all materials present register; the method can be relied on for both positive and negative information, and unexpected components do not escape detection.

IN THE literature on mass spectrometric analysis, numerous articles have stressed accuracy of quantitative determinations and the speed with which they can be attained (*6*, *7*, *10*, *12–16*). Little has been published, however, regarding the extensive use of the instrument in qualitative analysis. In this field the mass spectrometer possesses a considerable advantage over many chemical methods, in that every substance present is automatically registered in the mass spectrum. Thus, it is not necessary to make a distinctive test for each material to be determined, yet unexpected components do not escape detection.

The threshold of detection of various materials and the ease with which they may be identified vary, depending on several factors, including the concentration of the unknown and the identities, concentrations, and number of other materials present. The simplest cases are those in which each component of interest has several unicomponent peaks—i.e., peaks to which no other constituents ionize appreciably. A somewhat more difficult type of problem is the identification of an unknown, most of whose peaks also include contributions from other materials in the mixture. Some of these problems involving overlapping spectra can be solved by minor calculations. Others—such as those involving comparison to a standard—may often be solved by inspection of relative peak heights, particularly when only an indication of presence or absence of impurity, without identification of it, is required. As the complexity of the mixture increases, either qualitative or quantitative information becomes more difficult to obtain. Finally, if there are more overlapping components than major peaks, or if various calibrating spectra are unavailable, the problem may become impossible to solve completely without recourse to other methods. This fact detracts in no way from the value of the method in the large variety of problems which fall within its scope.

In either the simple or more complex cases of qualitative mass spectrometer analysis a ready knowledge of atomic weights, structural formulas, and normal stable isotope abundance ratios is essential, together with a familiarity with the characteristics of as many mass spectra as possible.

USE OF FORMULA AND ISOTOPE RATIOS

The peaks to be expected in the mass spectrum can be more or less predicted from the structural formula. Prediction is limited; it is well known that many materials exhibit substantial peaks which would not be expected purely from fracture of bonds as represented in the conventional formula. The peak at mass 29 in isobutane is an example; others are the 57 peak in 3,3-diethylpentane, or the 33 peak in isobutyl alcohol. In spite of the occurrence of these anomalous peaks, requiring rearrangement of the molecule (*3*, *7*, *9*, *17*), much useful information can be obtained by checking mass intervals in the mass spectrum against possible fragments predicted from the structural formula.

A series of peaks one unit apart, for instance, usually indicates successive removal of hydrogen atoms, and thus establishes the presence of that element in the unknown. Peaks 15 mass units apart strongly suggest a CH_3^+ ion. Among the many other intervals which may yield useful information are the following, which suggest the elements or radicals coupled with them: 14 (N or CH_2); 16 (O); 17 (OH); 29 (C_2H_5 or CHO); 32 (S); 35 (Cl); 41 (C_3H_5); 43 (C_3H_7); and 19 (F). A check of such intervals between peaks or groups of peaks in the spectrum of the unknowns is one of the first steps in qualitative work.

If the unknown compound ionizes to its parent mass (the mass computed from its formula, using the mass number of the most abundant isotope of each element present), the sizes of the one or two peaks at masses successively higher than the parent can in almost every case be predicted from the known isotope ratios, on the assumption of random distribution of the heavier atoms. The same type of prediction can be made for many fragments, although it is then somewhat less certain, because of preferential ionization (*2*) or formation of peaks requiring rearrangement (*9*). Even so, investigation of ratios of peaks 1, 2, or 3 mass units above a major peak is usually helpful at almost any point in the spectrum—for example, the appearance of one chlorine atom in either a parent or fragmented ion will result in peaks 2 masses apart in the ratio of 3 to 1. Thus, 2-chloropropane, parent mass 78, shows a peak at m/e 80 which is about $1/3$ of the 78. The same ratio holds in the fragment $CH_3 \cdot C \cdot CH \cdot Cl$ at masses 63 and 65, the heavier peak being due, of course, to presence of heavy chlorine. Purely on the basis of the 80/78 ratio, it is thus possible to distinguish between this compound and its mass isomer, benzene. For benzene, the 79/78 would be about 6.4% (6 carbons) and 80/78 would be about 0.2% (see Table I).

OBSERVED CHARACTERISTICS OF MASS SPECTRA

The greatest aid to qualitative analysis is a large library of mass spectra. Most laboratories have collected the numerous spectra in which they are most interested; many have made punch card files for ready reference. Now, an ever growing accumulation of spectral data is available through A.P.I. Project 44 (*1*).

Study of the numerous available patterns yields many useful generalizations regarding various classes of materials. Some observations useful in qualitative analysis of organic mixtures are listed below.

1. **General.** Almost no compounds show peaks above the

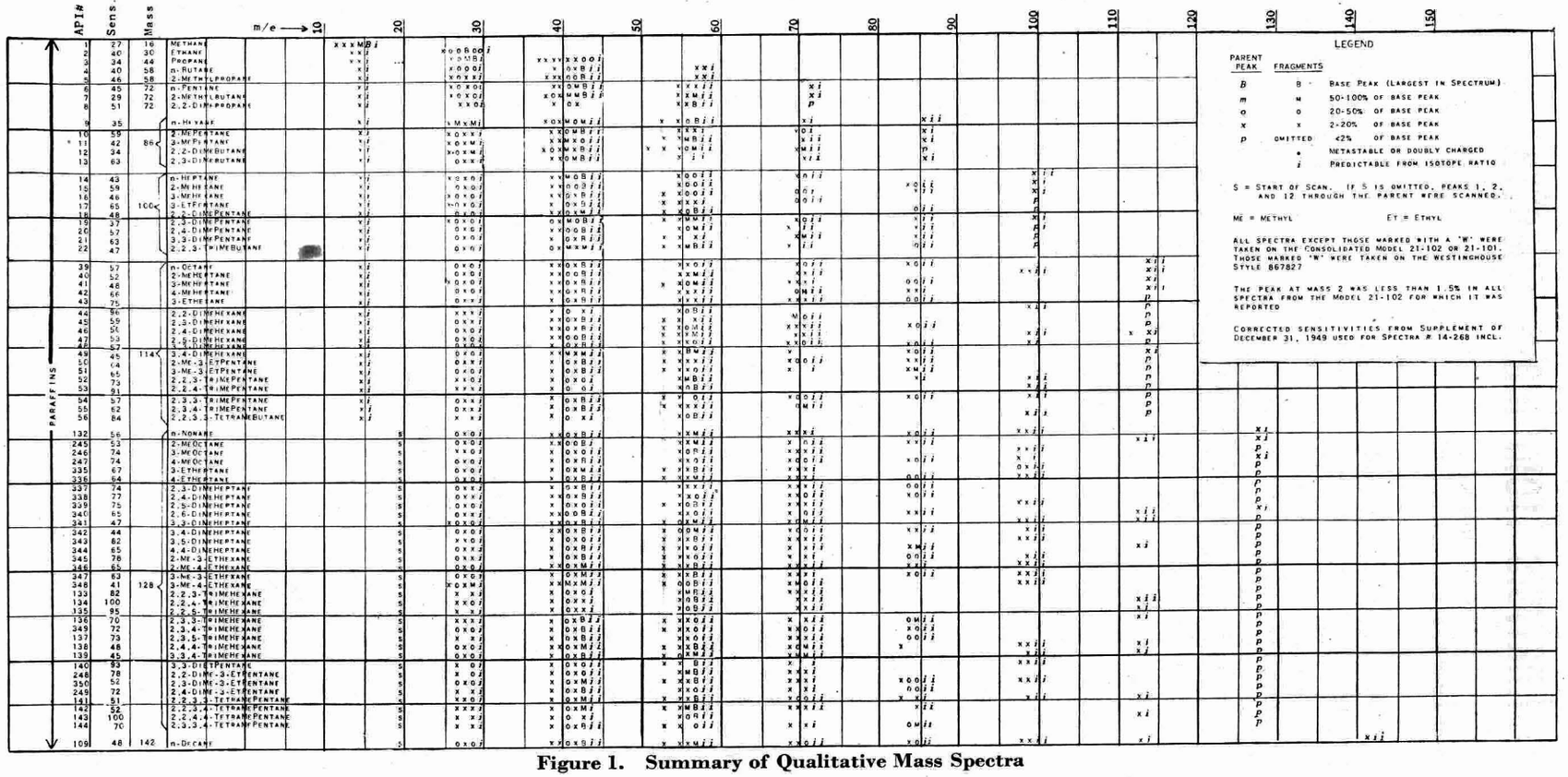


Figure 1. Summary of Qualitative Mass Spectra

Peaks in the mass spectra of 279 compounds are classified roughly according to magnitude, and charted opposite their correct mass numbers. For each compound, the molecular mass number, and an approximate sensitivity to the base peak, are also listed, together with the A.P.I. serial number of the spectrum if it was taken from the "Catalog of Mass Spectral Data" (*1*). Spectra charted from this catalog were submitted to A.P.I. by the following laboratories:

Laboratory	Serial Numbers of Spectra Used
National Bureau of Standards, Mass Spectrometry Laboratory, Washington, D. C.	1–26, 28–30, 32–56, 98–109, 128–144, 181–186, 188, 190–195, 202, 204, 206–209, 216–222, 224–228, 239–242, 244–276, 278, 280, 322, 328, 329, 331, 335–354, 359, and 400, all inclusive
Union Oil Co. of California, Wilmington, Calif.	72, 73, 74, 78–84, 86–90, 92, 93, 95, 96, and 97, all inclusive
Socony-Vacuum Laboratories, Paulsboro, N. J.	158–162, inclusive
Sinclair Refining Co., Harvey, Ill.	233–238, inclusive
Humble Oil and Refining Co., Baytown, Tex.	290, 292, 296, 300–303, 318–321, 322, 324, all inclusive
Consolidated Engineering Corp., Pasadena, Calif.	363–398, inclusive. Spectra included on chart but not in A.P.I. catalog: chloroethene, dichloromethane, 1,1-dichloroethene, trichloromethane, trichloroethene, 1,1,1-trichloroethane, carbon tetrachloride, sulfur dioxide, nitrogen, nitric oxide, nitromethane, nitroethane, 1-nitropropane, 2-nitropropane, water, 1,4-dioxane, and cyclohexanone.

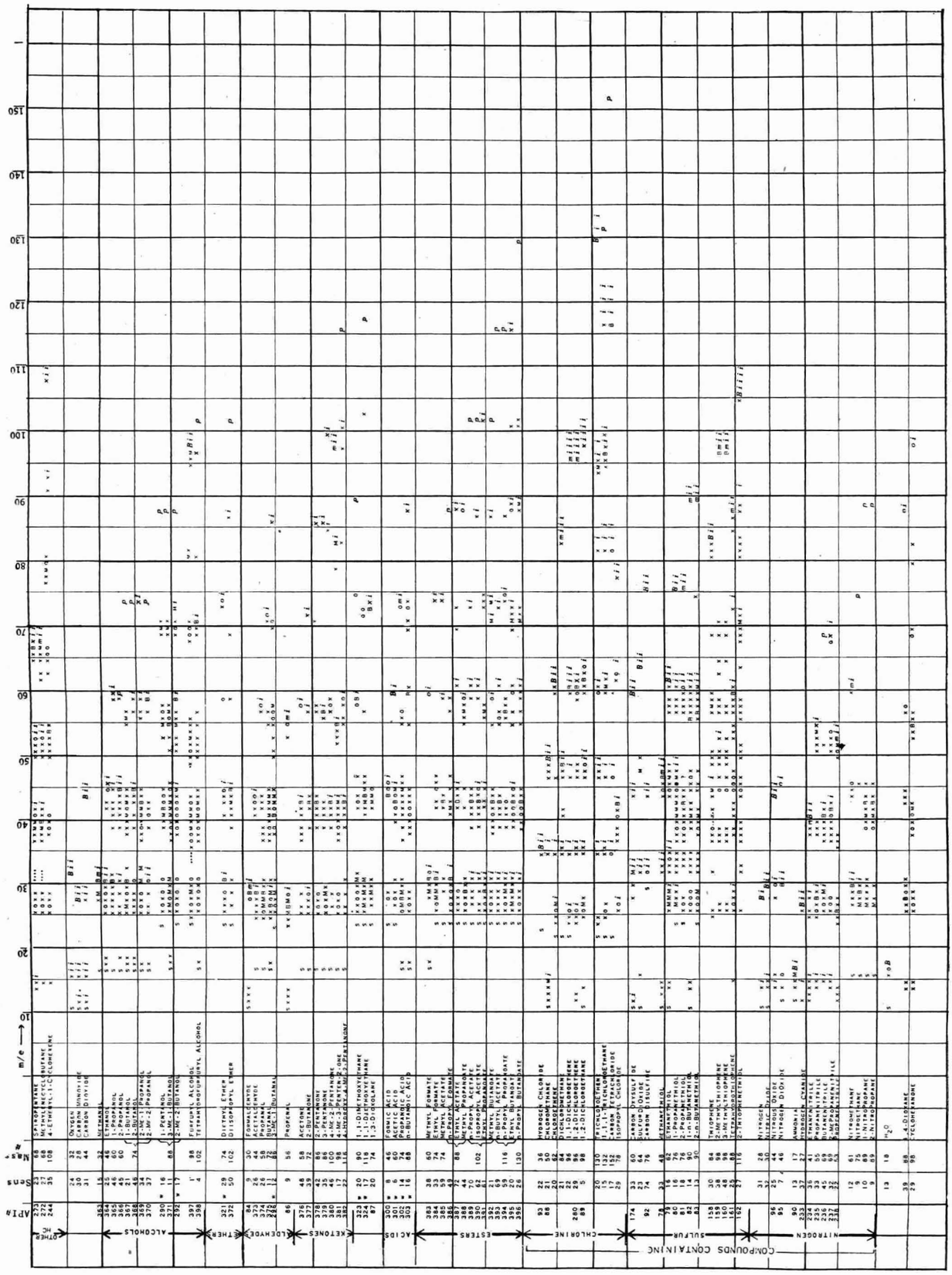
Parent Peak	Fragments	
B	B	Base peak (largest in spectrum)
m	M	50–100% of base peak
O	O	20– 50% of base peak
X	X	2– 20% of base peak
p	Omitted	2% of base peak
	. .	Metastable or doubly charged
	i	Peak due solely to isotope

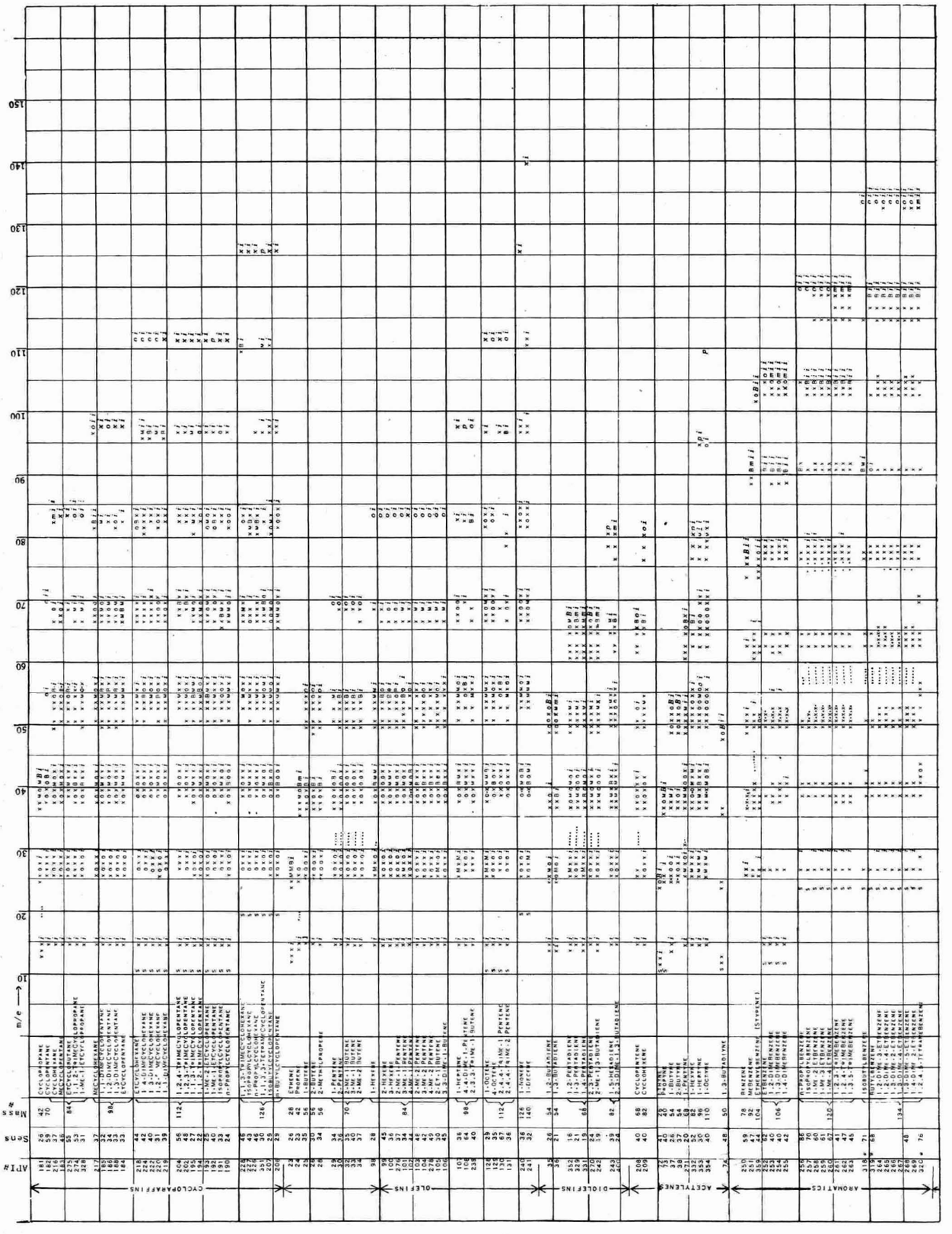
S = start of scan. If S is omitted, peaks 1, 2, and 12 through parent were scanned.
ME = methyl
ET = ethyl

All spectra except those marked with W were taken on Consolidated Model 21–102 or 21–101. Those marked W were taken on Westinghouse Style 867827.
Peak at mass 2 was less than 1.5% in all spectra from Model 21–102 for which it was reported.
Corrected sensitivities from supplement of Dec. 31, 1949, used for spectra 14–268, inclusive.

Because Figure 1 shows qualitative spectra, *i* is used for peaks very slightly over the computed isotope value as well as for those entirely from isotope. Some very small *i* peaks are omitted.

In the interest of clarity, only largest and/or most distinctive metastable and half-mass groups are included.




m/e
Mass #
Sens
API #
CYCLOPARAFFINS
OLEFINS
DIOLEFINS
ACETYLENES
AROMATICS
CYCLOPROPANE
CYCLOBUTANE
CYCLOPENTANE
METHYLCYCLOPROPANE
CYCLOHEXANE
METHYLCYCLOBUTANE
ETHYLCYCLOPROPANE
1,1-DIMETHYLCYCLOPENTANE
1,2-DIMETHYLCYCLOPENTANE
1,3-DIMETHYLCYCLOPENTANE
ETHYLCYCLOPENTANE
1,1,2-TRIMETHYLCYCLOPROPANE
METHYLCYCLOHEXANE
1,1,3-TRIMETHYLCYCLOPENTANE
1,2,4-TRIMETHYLCYCLOPENTANE
1,2,3-TRIMETHYLCYCLOPENTANE
1,1,2-TRIMETHYLCYCLOPENTANE
1-METHYL-2-ETHYLCYCLOPENTANE
1-METHYL-3-ETHYLCYCLOPENTANE
n-PROPYLCYCLOPENTANE
1,1,2,3-TETRAMETHYLCYCLOHEXANE
ISOPROPYLCYCLOHEXANE
n-PROPYLCYCLOHEXANE
ISOBUTYLCYCLOHEXANE
n-BUTYLCYCLOHEXANE
ETHENE
PROPENE
1-BUTENE
2-METHYLPROPENE
1-PENTENE
2-METHYL-1-BUTENE
3-METHYL-1-BUTENE
2-METHYL-2-BUTENE
1-HEXENE
2-HEXENE
3-METHYL-1-PENTENE
2-METHYL-1-PENTENE
4-METHYL-1-PENTENE
2-METHYL-2-PENTENE
3-METHYL-2-PENTENE
4-METHYL-2-PENTENE
2,3-DIMETHYL-1-BUTENE
3,3-DIMETHYL-1-BUTENE
1-HEPTENE
2-METHYL-1-HEXENE
2,3,3-TRIMETHYL-1-BUTENE
1-OCTENE
2,4,4-TRIMETHYL-1-PENTENE
2,4,4-TRIMETHYL-2-PENTENE
1-NONENE
1-DECENE
1,3-BUTADIENE
1,2-PENTADIENE
1,3-PENTADIENE
2,3-PENTADIENE
2-METHYL-1,3-BUTADIENE
1,5-HEXADIENE
1,3-DIMETHYL-1,2-BUTADIENE
CYCLOPENTENE
CYCLOHEXENE
DIACETYLENE
PROPYNE
1-BUTYNE
2-BUTYNE
1-PENTYNE
2-PENTYNE
1-HEXYNE
1-HEPTYNE
1-OCTYNE
1,3-BUTADIYNE
BENZENE
METHYLACETYLENE (PROPYNE)
ETHYNYLBENZENE (STYRENE)
TOLUENE
1,2-DIMETHYLBENZENE
ETHYLBENZENE
1,3-DIMETHYLBENZENE
1,4-DIMETHYLBENZENE
n-PROPYLBENZENE
ISOPROPYLBENZENE
1-METHYL-2-ETHYLBENZENE
1-METHYL-3-ETHYLBENZENE
1-METHYL-4-ETHYLBENZENE
1,2,3-TRIMETHYLBENZENE
1,2,4-TRIMETHYLBENZENE
1,3,5-TRIMETHYLBENZENE
ISOBUTYLBENZENE
1,2-DIETHYLBENZENE
1,3-DIETHYLBENZENE
1,4-DIETHYLBENZENE
1,2,3,4-TETRAMETHYLBENZENE

parent peak, except those predictable from the heavy isotopes of elements present.

Many spectra exhibit half-mass peaks (doubly charged ions) or metastable peaks (*4*, *5*) whose distinctive mass, size, and shape are very useful in identification. Among the helpful half-mass peaks are the 22 peak in the carbon dioxide spectrum, 20 in argon, and the half-mass group between 19 and 21 in the propane and propene spectra. A well-known metastable peak is that at m/e 31.9 in the *n*-butane spectrum. Some of the groups most distinctive in shape, and of large size, occur in the spectra of the aromatics—at 76 in benzene, 88 to 90 in toluene, and 58 to 59 in ethylbenzene (see Example 2).

2. **Hydrocarbons.** Paraffins do not ionize in substantial amounts to the parent peaks of lighter paraffins except for heavy isotope contributions—i.e., the 30, 44, 58, and 72 peaks in mass spectra of C_6 and heavier hydrocarbons are due primarily to $C_2H_5^+$, $C_3H_7^+$, $C_4H_9^+$, and $C_5H_{11}^+$ ions, respectively, each containing one C^{13} or one D (*17*). This fact makes feasible the ready detection of light paraffins in those of higher molecular weights. Light olefins or cycloparaffins cannot so readily be detected among heavier compounds of the same type, except for those 14 units below the parent. For example, mass 56 in the pentene spectra (parent = 70) is mostly isotope; 42, however, is not.

The isotopic distribution in hydrocarbons has been computed here on the assumption of random distribution of heavy atoms and no preferential ionization. Table I shows the distribution of ions of each atomic constitution (or formula) at successive masses, as percentages of the ions of principal mass, $(m/e)_p$. Principle mass is defined as that computed from the ion formula using C = 12 and H = 1. Thus, the fragment C_3H_7 has principal mass 43. Relative abundances of ions of formula C_3H_7 appearing at masses 44 = $(m/e_p + 1)$ (containing one C^{13} or one D), and 45 = $(m/e + 2)$ (containing 2 C^{13}'s, 1 C^{13} and 1 D, or 2 D's) are given in the table as 3.26 and 0.04%. The probability of appearance of either C^{13} or D, or both, was taken into consideration in computing Table I. These factors are useful for many quantitative as well as qualitative determinations, even though the assumption of no preferential ionization may sometimes fail (*2*). The ratio used for C^{13}/C^{12} was 1.04/98.96, and was obtained by averaging values from a number of repeated mass spectrometer runs of C_2 through C_6 paraffins, using only parent and heavier peaks for each compound. The common value, 0.00015/0.99985, was used for D/H (*11*).

Odd peaks are usually larger than even.

Above C_4, the parent peak is the largest peak in the parent C group in most hydrocarbon spectra. For example, for hexanes the 86 peak is the greatest in the group of peaks between C_6^+ and $C_6H_{14}^+$ (masses from 72 through 86). For C_5 and heavier few peaks appear in the parent group except the parent itself and, in a few cases, the peak immediately preceding it. Thus compounds differing 2, 4, etc., units in mass are readily recognized—for example, cycloparaffins or olefins in paraffins, or diolefins in materials of formula C_nH_{2n} or C_nH_{2n+2} (see Figure 1).

Straight-chain hydrocarbons have parent peaks but, as the carbon skeleton becomes more highly branched, the parent peak decreases. For example, peak 114 is inappreciable in 2,2,3,3-tetramethylbutane, but of useful size in *n*-octane (see Example 1).

C_4 and C_5 olefin isomers have very similar spectra. Beginning with C_6, greater differences among isomer patterns begin to appear.

3. **Oxygenated Materials.** Saturated alcohols, ethers, and esters have parent peaks 2 units above those of the paraffin of nearest parent mass—e.g., butanol is mass 74, *n*-pentane 72. Usually the spectra of oxygenated materials also contain peaks at odd masses, such as 31 or 45, to which hydrocarbons do not appreciably contribute. They are thus readily detected in the presence of hydrocarbons.

Aldehydes have the same molecular mass number or parent peak as hydrocarbons—e.g., propane and acetaldehyde both are of mass 44. However, peak ratios differ considerably, so that they are readily recognized when a few essentially unicomponent peaks are available for comparison.

Most of these observations may be verified and others deduced if Figure 1 is closely studied. This chart summarizes 279 mass spectra and indicates roughly the relative magnitudes of pattern coefficients. The legend in the figure explains the symbols used. Most of the spectra are taken from the A.P.I. "Catalog of Mass Spectral Data" (*1*), to which reference can be made for more refined coefficients.

Two examples are cited below to illustrate how the enumerated observations and others may be applied to yield valuable qualitative information on the mass spectra of unknown samples.

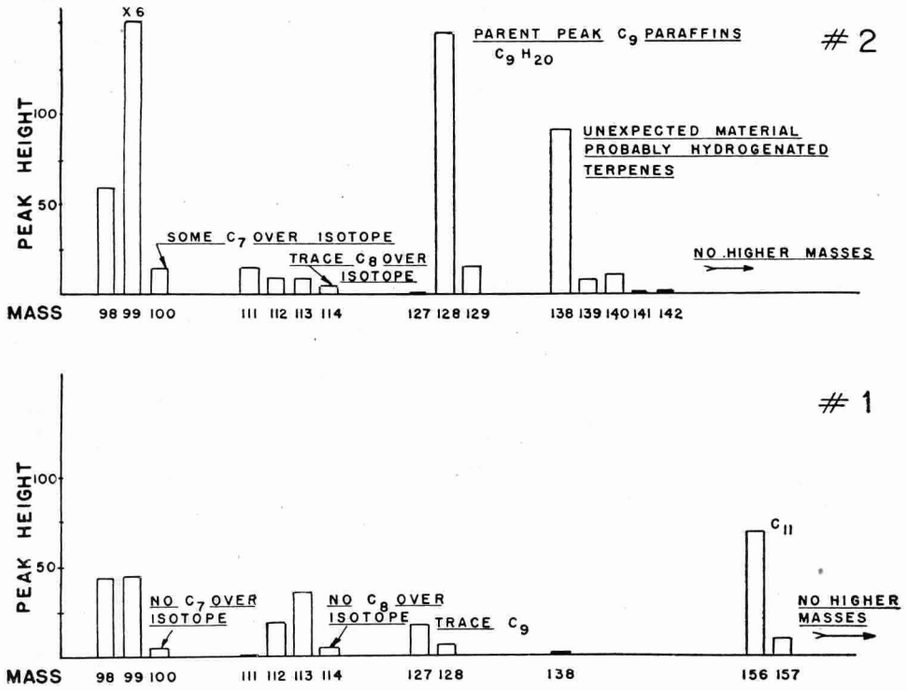


Figure 2. Schematic Mass Spectra of Narrow Pine Oil Fractions

1. Almost pure $C_{11}H_{24}$
2. Primarily C_9H_{20} with unexpected impurity showing at masses 138 to 142

Example 1. Purity Check. The first example illustrates a purity check of two closely fractionated pine oil samples (*8*), for which calibrations were not at the time available. Spectra obtained are

Table I. Isotope Correction Factors for C_1 through C_7 Hydrocarbons

This table was computed on the assumption of random distribution of heavy isotopes and no preferential ionization. The C^{13}/C^{12} ratio used was obtained by averaging ratios of (parent peak +1)/(parent peak) for numerous repeat runs of C_2-C_6 paraffins on the Consolidated Model 21-101 mass spectrometer.

$C^{13}/C^{12} = 0.0104/0.9896$
$D/H = 0.00015/0.99985$

Ion Formula[a]	$(m/e)_p$[b]	Percentage of Monoisotopic Peak at $(m/e)_p$ Which Appears at: $(m/e)_p + 1$	$(m/e)_p + 2$
C	12	1.05%	0
CH	13	1.07	1.6×10^{-4}%
CH_2	14	1.08	3.2×10^{-4}
CH_3	15	1.10	4.8×10^{-4}
CH_4	16	1.11	6.4×10^{-4}
C_2	24	2.10%	0.01
C_2H	25	2.12	0.01
C_2H_2	26	2.13	0.01
C_2H_3	27	2.15	0.01
C_2H_4	28	2.16	0.01
C_2H_5	29	2.18	0.01
C_2H_6	30	2.19	0.01
C_3	36	3.15%	0.03
C_3H	37	3.17	0.03
C_3H_2	38	3.18	0.03
C_3H_3	39	3.20	0.04
C_3H_4	40	3.21	0.04
C_3H_5	41	3.23	0.04
C_3H_6	42	3.24	0.04
C_3H_7	43	3.26	0.04
C_3H_8	44	3.27	0.04
C_4	48	4.20%	0.07
C_4H	49	4.22	0.07
C_4H_2	50	4.23	0.07
C_4H_3	51	4.25	0.07
C_4H_4	52	4.26	0.07
C_4H_5	53	4.28	0.07
C_4H_6	54	4.29	0.07
C_4H_7	55	4.31	0.07
C_4H_8	56	4.32	0.07
C_4H_9	57	4.34	0.07
C_4H_{10}	58	4.35	0.07

Ion Formula[a]	$(m/e)_p$[b]	Percentage of Monoisotopic Peak at $(m/e)_p$ Which Appears at: $(m/e)_p + 1$	$(m/e) + 2$
C_5	60	5.26%	0.11
C_5H	61	5.27	0.11
C_5H_2	62	5.28	0.11
C_5H_3	63	5.30	0.11
C_5H_4	64	5.32	0.11
C_5H_5	65	5.33	0.11
C_5H_6	66	5.34	0.12
C_5H_7	67	5.36	0.12
C_5H_8	68	5.38	0.12
C_5H_9	69	5.39	0.12
C_5H_{10}	70	5.40	0.12
C_5H_{11}	71	5.42	0.12
C_5H_{12}	72	5.44	0.12
C_6	72	6.31%	0.17
C_6H	73	6.32	0.17
C_6H_2	74	6.34	0.17
C_6H_3	75	6.35	0.17
C_6H_4	76	6.37	0.17
C_6H_5	77	6.38	0.17
C_6H_6	78	6.40	0.17
C_6H_7	79	6.41	0.17
C_6H_8	80	6.43	0.17
C_6H_9	81	6.44	0.17
C_6H_{10}	82	6.46	0.18
C_6H_{11}	83	6.47	0.18
C_6H_{12}	84	6.49	0.18
C_6H_{13}	85	6.50	0.18
C_6H_{14}	86	6.52	0.18

Ion Formula	$(m/e)_p$	Percentage of Monoisotopic Peak at $(m/e)_p$ Which Appears at: $(m/e)_p + 1$	$(m/e)_p + 2$	$(m/e)_p + 3$
C_7	84	7.36%	0.23	0.004
C_7H	85	7.37	0.23	0.004
C_7H_2	86	7.39	0.23	0.004
C_7H_3	87	7.40	0.24	0.004
C_7H_4	88	7.42	0.24	0.004
C_7H_5	89	7.43	0.24	0.004
C_7H_6	90	7.45	0.24	0.004
C_7H_7	91	7.46	0.24	0.004
C_7H_8	92	7.48	0.24	0.004
C_7H_9	93	7.49	0.24	0.004
C_7H_{10}	94	7.51	0.24	0.004
C_7H_{11}	95	7.52	0.24	0.004
C_7H_{12}	96	7.54	0.24	0.004
C_7H_{13}	97	7.55	0.25	0.005
C_7H_{14}	98	7.57	0.25	0.005
C_7H_{15}	99	7.58	0.25	0.005
C_7H_{16}	100	7.60	0.25	0.005

[a] Chemical formula of monoisotopic ion.
[b] Principal m/e of ion (C = 12, H = 1).

Table II. Mass Spectra of Cyclohexanone, Toluene, 1,4-Dioxane, and Acetone

MS. Consolidated Model 21-102
Mass range. 28, 37-99
Electron current. 9 μa.
Electron accelerating voltage. 70 volts
Ionization chamber temperature. 250° C.

m/e	Cyclohexanone	Toluene	1,4-Dioxane	Acetone
28	12.5	0.5	100	1.9
37	1.8	2.5	...	2.2
38	4.1	5.0	...	2.3
39	22.4	18.7	0.2	3.8
40	6.9	2.1	0.2	0.8
41	32.7	2.1	0.7	2.2
42	81.6	0.2	1.9	7.1
43	12.2	1.8	10.9	100
44	1.2	1.1	2.4	2.3
45	0.2	5.7	3.0	0.2
46	...	4.0	...	...
50	1.7	5.9	...	...
51	1.9	9.6	...	...
52	0.7	2.4	...	0.1
53	3.0	1.2	...	0.4
54	7.5	..	...	...
55	100	..	...	0.3
56	11.5	..	0.4	...
57	2.1	..	5.7	0.8
58	0.1	..	23.6	27.8
59	...	..	1.1	0.9
60	...	0.3	0.1	...
61	0.1	2.1	0.1	...
62	0.3	4.2	0.1	..
63	0.5	8.6	0.1	..
64	..	2.1	..	..
65	0.4	13.1	..	..
66	0.2	1.7	..	..
69	24.3	...	..	..
70	18.8	...	..	..
71	1.7	...	..	..
77	..	1.2	..	..
80	3.3	...	..	..
83	6.1	...	...	..
87	..	0.5	1.9	..
88	..	0.2	28.3	..
89	..	3.6	1.3	..
90	..	8.2	0.2	..
91	..	100	..	..
92	..	74.8	..	..
93	..	5.4	..	..
97	1.7	...	..	..
98	28.9	...	..	..
99	2.0	...	..	..
Sensitivities. 29 div./μ (n − C_4 43 = 40 div./μ)		47	39	48

schematically represented in Figure 2. Spectrum 1 is nearly pure $C_{11}H_{24}$ (m/e = 156). The peak at m/e 157 is due to presence of heavy isotope. No straight-chain $C_{11}H_{22}$ or $C_{11}H_{20}$ is present. The peak at 138 is probably a minute impurity. Peak 128 is slightly greater than the peak predicted from isotope coefficients of Table I, so that a trace of C_9 is present. The large parent peak (m/e = 156) indicates straight-chain or nearly straight-chain C_{11}'s rather than the highly branched compounds. The sample is thus shown to be practically pure C_{11} paraffins, the absence of peaks between the expected C groups attesting absence of many possible contaminating materials—naphthenes and aromatics, to mention only two.

Spectrum 2 was supposed to be a comparably pure C_9. The substantial peak at 128 indicates a large amount of C_9 paraffin, again not highly branched. Small excesses over isotope at 100 and 114 indicate traces of C_7 and C_8. The major surprise, however, was the presence of substantial peaks from 138 to 142. There is no doubt of their presence. From other considerations (*8*) they are tentatively identified as hydrogenated terpenes.

Thus, valuable information regarding presence or absence of contaminants was obtained from mere inspection of the record, without recourse to specific calibrations.

Example 2. Identification of Unknown Solvents. Example 2 shows the spectrum of a mixture of organic liquids. None of the components had been identified prior to running the mass spectrum. The presence of toluene was obvious at first glance at the spectrum, because of the distinctive metastable group around mass 90 and the half-mass peaks at masses 43 through 46. These are shown in Figure 3, in which the lower spectrum was obtained from the mixture, the upper from pure calibrating toluene. The records are from a standard Consolidated Model 21-102 mass spectrometer, with three galvanometer traces

masked out, leaving only one showing to facilitate reproduction. The records on this instrument are taken at a constant speed regardless of the peak size, so that the mass scale (abscissa) is the same for all records. Thus peaks in the toluene spectrum can be directly compared to those of the mixture spectrum which are directly below them.

The 58/43 ratio in the mixture spectrum suggested that acetone rather than normal or isobutane was the chief contributor to these peaks. The peaks at masses 88 and 98 were assumed to be due to two separate materials, as the 10 unit mass difference could not be explained by any common radical. The spectra had been run to mass 150, and no peaks found above mass 98. Even so, it did not necessarily follow that peaks 98 and 88 were the parent peaks of the substances remaining to be identified, for not all materials ionize to the parent mass. The initial assumption was made, however, that these were the molecular mass numbers.

The 83 peak was associated with the peak at mass 98 because of the 15-unit mass difference usually associated with CH_3^+. Similarly, the peak at mass 69 was most logically ascribed to the mass 98 material because it represented loss of the fragment $C_2H_5^+$ (*29*). Similarly, the large peak at 55 was probably due to the mass 98 substance. The ratio of peaks 99/98 shows that not more than 6 carbons were present, so that C_7 olefins and cycloparaffins were eliminated. The small 100/98 ratio (0.5% as determined on more sensitive traces not shown here) eliminated chlorine and sulfur as possibilities and suggested the presence of oxygen. Comparison with spectra of C_6 oxygenated materials identified this material as cyclohexanone.

The mass 88 material was now known to contribute to peak 31, because none of the other constituents ionized to that mass to an appreciable extent. When acetone, toluene, and cyclohexanone contributions were subtracted from the mixture spectrum, the remaining peaks were identified by their relative sizes, as those of dioxane. Spectra of all four solvents are shown in Table II.

Negligible residuals remained after quantitative determination of the four components, showing that the constituents had been correctly identified.

SUMMARY

The fact that all materials present in concentrations above their threshold of detection automatically indicate their presence on the mass spectrum is particularly useful in establishing the presence or absence of compounds, whether or not they were previously anticipated.

Of the many devices and techniques used by the mass spectroscopist to extract qualitative information from the mass spectrum, the following have been discussed and some of them illustrated:

Check of mass intervals to identify fragments and elements probably present.

Observation of peak-free regions to establish absence of materials contributing to those portions of the record.

Comparison of relative sizes of major peaks and those immediately following with those expected from heavy isotopes, to ascertain probable presence or absence of elements with stable isotopes.

Recognition of characteristic peak shapes and distinctive peak groupings on the record to identify constituents—e.g., metastable and half-mass peaks.

Use of general knowledge of mass spectral characteristics of various classes of compounds to identify types of materials present. The observations cited can be supplemented by study of Figure 1. This table also facilitates the preliminary screen-

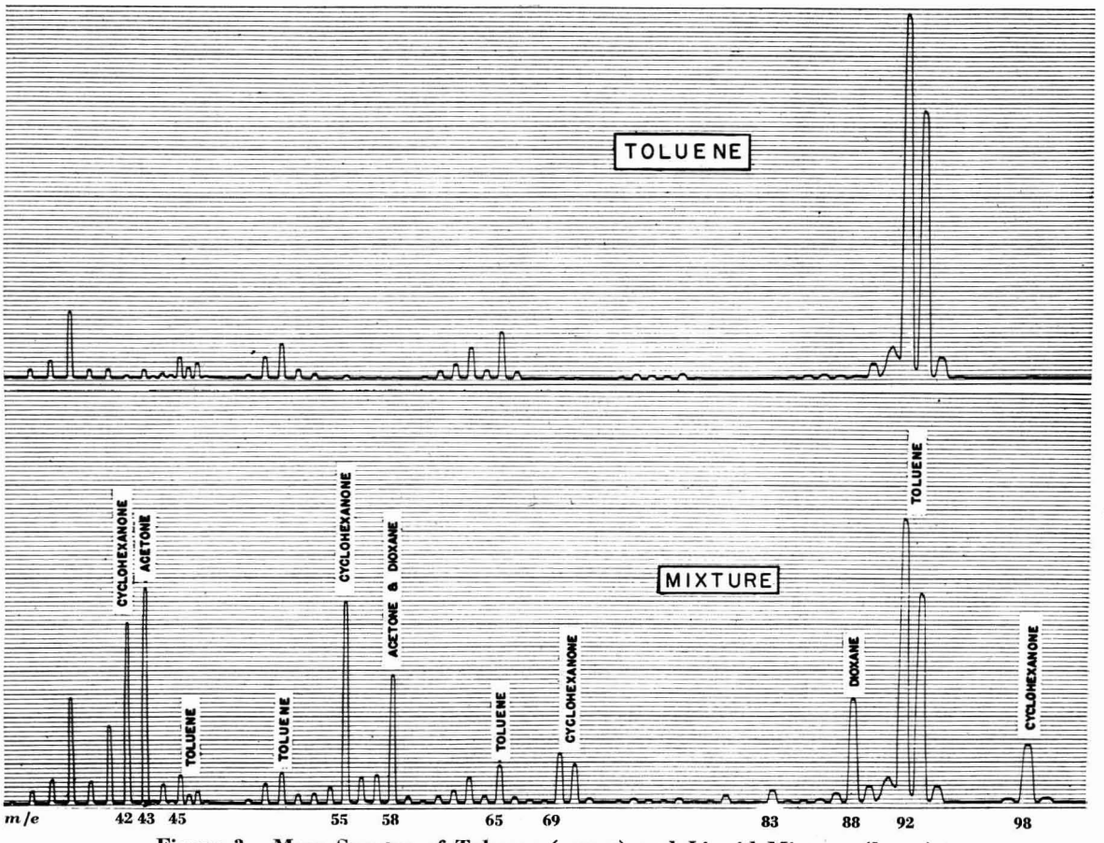


Figure 3. Mass Spectra of Toluene (*upper*) and Liquid Mixture (*lower*)

Record obtained on Consolidated Model 21-102 mass spectrometer, with traces 1, 3, and 30 masked off. Half-mass peaks at 44 to 46 and metastable at 90 immediately identify toluene in mixture. Major peaks contributed by three other components of mixture are also marked.

ing check of the peaks to be identified against a number of mass spectra.

ACKNOWLEDGMENT

The constructive suggestions offered by H. W. Washburn and A. P. Gifford were most helpful and are much appreciated.

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Effect of Finite Slit Width on Infrared Absorption Measurements

A. R. PHILPOTTS, WILLIAM THAIN, AND P. G. SMITH
The Distillers Co., Ltd., Great Burgh, Epsom, Surrey, England

Extinction coefficients in the infrared vary with the resolving power—i.e., they depend on the optical arrangement of a spectrometer and on the slit width used. This work attempts to find practically and (within the limits of the assumptions made) theoretically the extent of this variation. Plots of absorption against concentration for solutions of three hydrocarbons were made at different slit settings, using a Perkin-Elmer Model 12B spectrometer. The variations in slope and the deviations from linearity were found to fit (formally at least) the theoretical explanation given. The magnitude of the effect shows that spectroscopists must be very careful when using extinction coefficients determined under optical conditions not identical with those of the analysis. A method of correlating data taken at different slit settings on the same spectrometer in the same state of optical adjustment is given. It is hoped that the correction of extinction coefficients to "infinite resolving power" suggested will enable extinction coefficient measurements to be used on all spectrometers.

ESTIMATIONS by absorption spectrophotometry are greatly simplified when deviations from the Beer-Lambert law are smaller than experimental error, especially when multicomponent mixtures can be analyzed by the method of solving linear simultaneous equations. The dependence of extinction coefficients on instrumental conditions (a great difficulty of infrared spectroscopy) is also bound up with the applicability of the law. Much work has therefore been devoted to the investigation of deviations.

Failures of the law due to the inherent properties—e.g., intermolecular forces, etc.—of the sample in question are to some extent unavoidable, but it should be possible to correct for or at least calculate the magnitude of instrumental limitations. These chiefly concern scattered radiation in the monochromator and the effect of finite slit width. Methods of dealing with the first have been reported (*4*). While attention (*6*, *10*) has recently been drawn to the second, and the general principles (*3*, *7*) have been laid down, there has been no discussion of the magnitude of the errors caused when conventional infrared spectrometers are used.

The case of the area of absorption bands has been considered (*3*, *11*). While this manuscript was in the course of preparation, applications to ultraviolet problems were published by Eberhardt (*2*).

Whether single- or double-beam spectroscopy is employed, the observed density is always the logarithm of the ratio of incident to transmitted intensity, each intensity being integrated over the pass band of the monochromator. The error is introduced by assuming that the density obtained in this way is identical with the true density at the central wave length. The magnitude of the error at the maximum of an absorption band depends on the relation of the pass band width to the true shape of the band. It is very difficult to obtain the shape of a band at infinite resolving power and the pass band of a monochromator depends on such indefinite quantities as image aberration and line-up, as well as the (possibly) calculable geometric slit width, diffraction effect, and image curvature.

The best method of procedure seems to be:

Calculation of errors in terms of an idealized absorption band and spectrometer response

Evaluation of parameters in this mathematical analysis by comparison with observed deviation from Beer's law

Assessment of the value of the analysis by comparison of the parameters with observable quantities

MATHEMATICAL ANALYSIS OF IDEAL CASE

The Beer-Lambert law is usually stated in the form

$$I = I_0\, 10^{-d}$$

where I_0 = intensity of incident radiation, I = intensity of transmitted radiation, and d = optical density. (The term "optical density" is used rather than "absorbance" because both logarithmic bases are employed.) This statement is used in the discussion of experimental data in the next section, but for ease of manipulation let us use here the natural logarithm, so that

$$I = I_1\, e^{-d'}$$

where 0.4343 d′ = d.

The transmission function of a monochromator depends on a number of calculable and incalculable factors. It is therefore proposed in this exploratory survey to use the simplest form (*5*)—namely, that this function is constant between two limiting wave lengths (determined by the wave length and slit settings of the spectrometer) and is zero outside these.

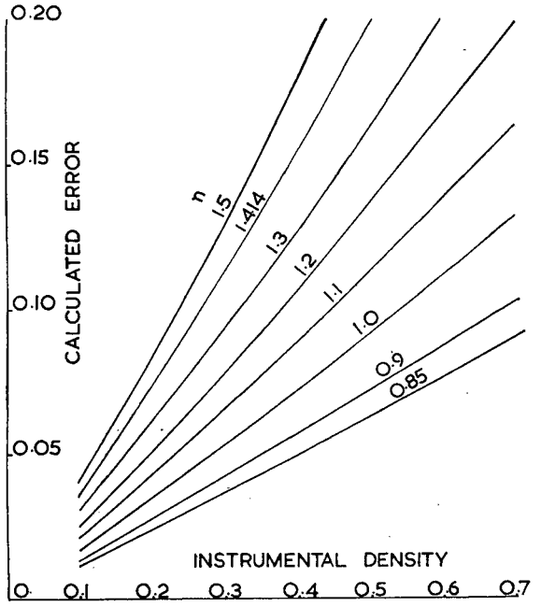

Figure 1. Graphs of Calculated Error [0.4343 log (*z*)] against Instrumental Density {0.4343 [d_m — log (*z*)]} for Values of *n* Shown

Suppose the monochromator is set at λ_0 and passes the wave band between $\lambda_0 - s$ and $\lambda_0 + s$ where $2s$ is defined as the "equivalent slit width." If the monochromator is used to measure the optical density of a material with a maximum absorption at λ_0, the observed value, $d'_{obsd.}$, is then given by

$$d'_{obsd.} = \log \int_{\lambda_0 - s}^{\lambda_0 + s} I_0 d\lambda - \log \int_{\lambda_0 - s}^{\lambda_0 + s} I d\lambda$$

Let the true density (at infinite resolving power) at any wave length, λ, be d′

$$d'_{obsd.} = \log \int_{\lambda_0 - s}^{\lambda_0 + s} I_0 d\lambda - \log \int_{\lambda_0 - s}^{\lambda_0 + s} I_0\, e^{-d'}\, d\lambda$$

If I_0 is constant over the range $\lambda_0 \pm s$, the first term can be integrated and the expression reduces to

$$d'_{obsd.} = -\log \frac{1}{2s} \int_{\lambda_0 - s}^{\lambda_0 + s} e^{-d'}\, d\lambda$$

This integral can be evaluated only if some relationship between d′ and λ is known. It has been shown (*9*) that in solution single absorption bands approximate to a simple error function in shape even at high resolving power. Let us therefore assume that at infinite resolving power

$$d' = d'_m\, e^{-\frac{1}{2}\left(\frac{\lambda - \lambda_0}{l}\right)^2}$$

Here d'_m is the optical density at the maximum and l is the quantity which determines the "peakedness" of the band. In fact, $2l$ is the width of the band when $d' = 0.6064\, d'_m$—i.e., the distance between the points of inflection—and is used here as a measure of band width rather than the more conventional width when $d' = 0.5\, d'_m$ (the "half band width").

To simplify, let $x = \dfrac{\lambda - \lambda_0}{l}$

and let $n = \dfrac{s}{l} = \dfrac{\text{equivalent slit width}}{\text{band width}}$

$$d'_{obsd.} = -\log \frac{l}{2n} \int_{-n}^{+n} \exp\left\{-d'_m\, e^{-x^2/2}\right\} dx \qquad (1)$$

Now $\exp\left\{-d'_m\, e^{-x^2/2}\right\} =$

$$\exp\left\{-d'_m \left(1 - \frac{x^2}{2} + \frac{1}{2!}\frac{x^4}{4} - \frac{1}{3!}\frac{x^6}{8} + \frac{1}{4!}\frac{x^8}{16}\cdots\right)\right\} =$$

$$e^{-d'_m} \times e^{+d'_m \frac{x^2}{2}} \times e^{-d'_m \frac{x^4}{8}} \times e^{+d'_m \frac{x^6}{48}} \times e^{-d'_m \frac{x^8}{384}} \ldots.$$

Expanding the exponentials again, multiplying out, and collecting,

$$\exp\left\{-d'_m\, e^{-x^2/2}\right\} = e^{-d'_m}\left\{1 + \frac{d'_m}{2}x^2 + \frac{d'_m(d'_m - 1)}{8}x^4 + \frac{d'_m(d'^2_m - 3d'_m + 1)}{48}x^6 \ldots\ldots\right\}$$

Integrating Equation 1 and using the first five terms in the expansion

$$d'_{obsd.} = d'_m - \log\left\{1 + \frac{d'_m}{6}n^2 + \frac{d'_m(d'_m - 1)}{40}n^4 + \frac{d'_m(d'^2_m - 3d'_m + 1)}{336}n^6 + \frac{d'_m(d'^3_m - 6d'^2_m + 7d'_m - 1)}{2688}n^8\right\}$$

$$d'_{obsd.} = d'_m - \log(z) \qquad (2)$$

From Equation 2 the true density can be calculated for any observed value if the ratio, n, is known. The convergence of the series depends on the magnitudes of both n and d'_m. It was found that with $n > 1.55$ the series did not converge rapidly enough for densities of practical interest.

The best method of handling the rather formidable expression proved to be the plotting of the calculated error, 0.4343 log (z), against the corresponding instrumental density, $d_{obsd.} = 0.4343\,[d'_m - \log(z)]$, for values of n. Some examples are shown in Figure 1, though in practice a large scale graph with more n values was used.

It is believed that even with modern infrared spectrometers n is rarely less than 1 and often much greater. Table I shows examples of the calculated magnitude of the effect at typical values of n.

It can be seen that a Beer's law curve determined when $n = 1$ will give a nearly straight plot with slope about 15% less than the true calibration coefficient, while one determined with $n = 1.414$ will have appreciable curvature.

Table I. Calculated Density

True Density	Instrumental Density	
	$n = 1$	$n = 1.414$
0.217	0.185	0.160
0.304	0.258	0.222
0.434	0.368	0.315
0.565	0.478	0.408
0.651	0.550	0.468
0.869	0.730	0.617

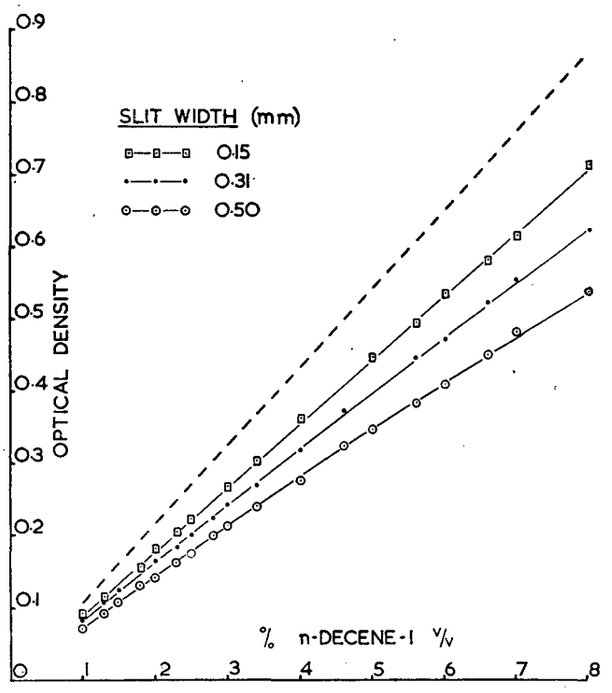

Figure 2. Experimental Calibration Lines for 913 Cm.$^{-1}$ Band of *n*-1-Decene at Different Slit Widths

Broken line, corrected calibration line

The limiting cases when n is small and when the density is small are of interest.

When *n* is small.

$$d'_{obsd.} \approx d'_m - \frac{d'_m}{6} n^2 = d'_m \left(1 - \frac{n^2}{6}\right)$$

Multiplying by 0.4343 and dividing by the concentration:

Observed calibration coefficient =

$$\text{true calibration coefficient} \times \left(1 - \frac{n^2}{6}\right) \quad (3)$$

When d'_m is small.

$$d'_{obsd.} \approx d'_m - \log \left\{1 + d'_m \left(\frac{n^2}{6} - \frac{n^4}{40} + \frac{n^6}{336} - \frac{n^8}{2688} \cdots\right)\right\}$$

$$\approx d'_m \left(1 - \frac{n^2}{6} + \frac{n^4}{40} - \frac{n^6}{336} + \frac{n^8}{2688}\right)$$

Multiplying by 0.4343 and dividing by the concentration:

Slope of tangent at origin to observed curve =

$$\text{true calibration coefficient} \times \left(1 - \frac{n^2}{6} + \frac{n^4}{40} - \frac{n^6}{336} + \frac{n^8}{2688} \cdots\right) \quad (4)$$

EXPERIMENTAL VERIFICATION

In order to reduce deviations from the Beer-Lambert law due to effects other than the one under discussion, careful choice of instrumental conditions and of test substances was required.

Scattered Radiation. If the scattered radiation at λ_0 is not absorbed at its true wave lengths by the test substance, the method of Hogness *et al.* gives an accurate correction. It is necessary therefore to work with the strongest band in the spectrum of the test substance.

Chemical Effects. To prevent deviations due to intermolecular effects, the test substance must be non-polar and inert solvents must be used.

Solvents. It is preferable to work in solution, so that the optical density can be varied by varying the concentration. This variation means an alteration in the amount of solvent in the radiation path which will influence the shape of the density-concentration curve unless the solutions are very dilute or the solvent is completely transparent.

Shape of Band. The absorption band used must have no obvious asymmetry, in order to give the theory a reasonable test.

These conditions (together with availability of materials) limit the possible bands to δ C-H frequencies in hydrocarbons and the solvents to cyclohexane and carbon tetrachloride. Cyclohexane is particularly suitable, because it is completely transparent at long wave lengths. Measurements were made using the 913 cm.$^{-1}$ band of *n*-1-decene, the 829 cm.$^{-1}$ band of *p*-diisopropylbenzene, and the 720 cm.$^{-1}$ band of cetane. Some experiments were carried out with the 728 cm.$^{-1}$ toluene band, but it proved to be too narrow (with the resolving power available) to give manageable values of n.

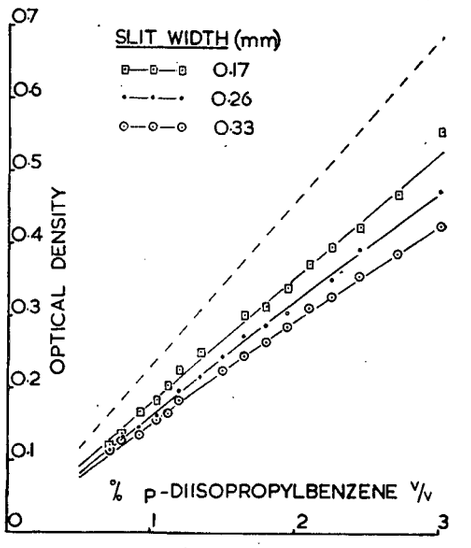

Figure 3. Experimental Calibration Lines for 829 Cm.$^{-1}$ Band of *p*-Diisopropyl Benzene at Different Slit Widths

Broken line, corrected calibration line

Experimental Procedure. A Perkin-Elmer 12B infrared spectrometer was used. The attenuator system was found extremely useful to fulfill the double condition of constancy of slit setting and constancy of I_0. The same rock salt cell (approximately 0.1 mm. thick) was used for all experiments. A series of solutions was made up in each case and short sections of the spectra were run alternately with solution and solvent in the cell. Zeros were taken at the beginning and end of each section with a lithium fluoride shutter. The scattered radiation correction was found by observing the fraction of incident radiation passed by the shutter but not by a strong enough solution to give an appreciably flat-topped band. This condition could not be fulfilled with cetane, so the correction was assumed to be the same at 720 cm.$^{-1}$ as at 728 cm.$^{-1}$, where it was determined with toluene.

The density was calculated as $\log \frac{B - S}{T - S}$ where B is the distance between trace and zero at the wave length required with the solvent in the cell, T the distance with solution in the cell, and S the scattered radiation correction. A mean value for B was obtained from readings before and after the measurement of T.

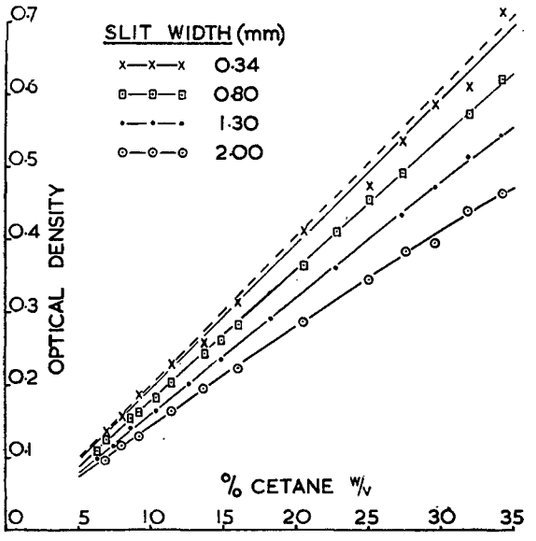

Figure 4. Experimental Calibration Lines for 720 Cm.$^{-1}$ Band of Cetane at Different Slit Widths

Broken line, corrected calibration line

The experimental Beer's law plots at various slit widths are shown in Figures 2 to 4.

Having obtained a series of observed densities for one slit setting, it was possible to find n by applying corrections such as those in Figure 1. Corrections from curves for different values of n were applied in turn until, at a particular value of n, the quantity $\frac{d_{obsd.} + 0.4343 \log (z)}{C}$ was constant within experimental error for all concentrations—i.e., until the corrected density gave a linear Beer's law plot.

It was found that the straight lines obtained by correcting the curves obtained at different slit widths for the same substance had the same gradient, in accordance with the theory. This "corrected calibration line" is indicated on Figures 2 to 4 as a broken line. When n is small the experimental plot is linear and the value of n can be obtained only if the corrected calibration coefficient has been found using wider slits. In that case the ratio of experimental to corrected calibration coefficient is $\left(1 - \frac{n^2}{6}\right)$ to 1 by Equation 3. This method was, in fact, used to find the low value of n for cetane, and the figure is only approximate because the ratio is nearly unity. The high values of n were determined approximately by comparing the slopes of the tangents at the origin of the curves in question with the corrected calibration coefficient (see Equation 4).

A summary of the results is given as Table II. In order to show the consistency of the results and also to demonstrate the curvature of the observed plots, the value of observed density divided by concentration is given for all determinations on cetane in Figure 5. The variation of observed calibration coefficient with concentration (and therefore with density) and with slit width is apparent. The top part of Figure 5 shows how the calibration coefficient after correction is constant within experimental error.

The values of the standard deviation in Table II are worthy of note. The high values for the first set of points in the case of *p*-diisopropylbenzene and of cetane are due to the unsteadiness of the recorded spectra at maximum amplifier gain. The last set for these two substances also shows high deviation, since the applied correction is high which emphasizes errors in determination—a high value receiving a high correction and a low value a low one.

Table II. Summary of Results

Substance	Slit Width, Mm.	No. of Observations	n	Corrected Calibration Coefficient: Mean (density per 1%)[a]	Corrected Calibration Coefficient: Standard deviation
n-1-Decene, 913 cm.$^{-1}$	0.11	3	Ca. 1.07	...	
	0.15	16	1.15	0.1107	0.0016
	0.31	17	1.41	0.1109	0.0014
	0.50	18	Ca. 1.85	...	
p-Diisopropyl benzene, 829 cm.$^{-1}$	0.17	16	1.25	0.2286	0.0079
	0.26	13	1.50	0.2296	0.0041
	0.33	16	Ca. 1.8	...	
Cetane, 720 cm.$^{-1}$	0.265	12	Ca. 0.34	0.02034	0.00061
	0.80	15	0.85	0.02031	0.00024
	1.30	12	1.20	0.02032	0.00026
	2.00	12	1.53	0.02032	0.00035

[a] For *n*-1-decene and *p*-diisopropyl benzene, 1% means 1 ml. per 100 ml. of solution, and for cetane 1 gram per 100 ml. of solution.

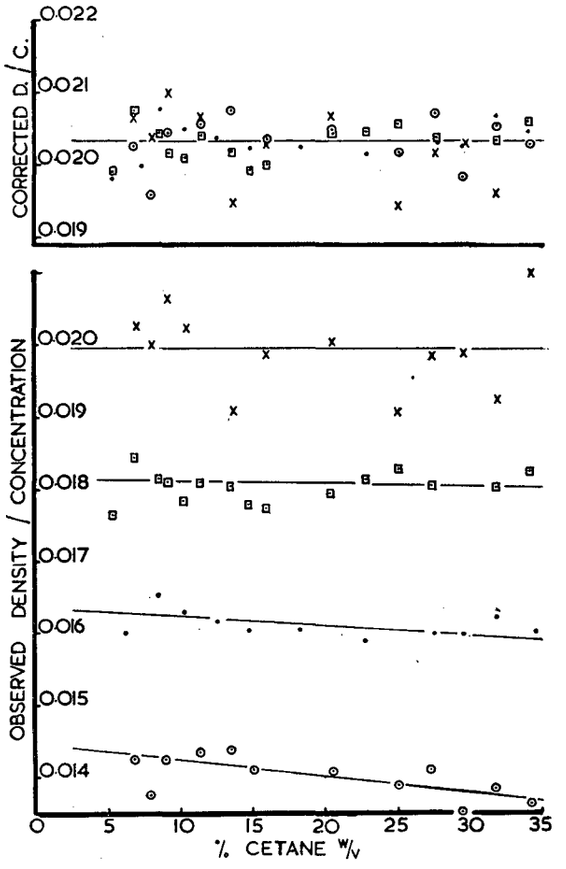

Figure 5. Calibration Coefficient

Upper. **Constant calibration coefficient obtained by correcting observed density before evaluating coefficient**

Lower. **Variation of calibration coefficient (observed density/concentration) with slit width and concentration for 720 cm.$^{-1}$ band of cetane**

MEANING OF OBSERVED VALUES OF *n*

Having found that the experimental points agree formally with Equation 1, the physical significance of the parameters found (d_m and n) must be considered. Ideally, the bands should now be remeasured, using a very high resolving power instrument—e.g., a grating spectrometer—and d_m and the band width $2l$ found directly. As neither the instrument nor the data are available, a less direct approach must be used.

Table III. Slit Width

Substance	Slit Width, Mm.	n	Band Width, Cm.$^{-1}$	Equivalent Slit Width, Cm.$^{-1}$	Effective Slit Width, Cm.$^{-1}$	Difference Cm.$^{-1}$
n-1-Decene, 913 cm^{-1}	0.11	Ca. 1.07	6.2	6.6	2.3	4.3
	0.15	1.15		7.1	2.8	4.3
	0.31	1.41		8.7	4.8	3.9
	0.50	Ca. 1.85		11.5	7.5	4.0
p-Diisopropyl benzene 827 cm.$^{-1}$	0.17	1.25	2.8	3.5	2.3	1.2
	0.26	1.50		4.2	3.1	1.1
	0.33	Ca. 1.8		5.0	3.8	1.2
Cetane, 720 cm.$^{-1}$	0.265	Ca. 0.34	9.5	3.2	1.7	1.5
	0.80	0.85		8.1	4.1	4.0
	1.30	1.20		11.4	6.4	5.0
	2.00	1.53		14.5	9.7	4.8

The area under a density curve for a single band is approximately independent of slit width (*7*) and the area per unit concentration is approximately independent of concentration (*8*). The area per unit concentration using a fairly narrow slit therefore gives a measure of the area per unit concentration at infinite resolving power (*10*). The area under the curve is $\sqrt{2\pi} \times$ height $\times\ l$ where l is half the distance between the points of inflection—i.e., half the band width at 0.6064 of maximum height. It follows that the quantity $\frac{\text{density} \times \text{band width}}{\text{concentration}}$ is constant and approximately independent of resolving power. As we already know $\frac{\text{density}}{\text{concentration}}$ for the ideal case, the value of the constant from observed data will enable us to find $2l$ at infinite resolving power.

Four widely distributed points on each of the observed density-concentration curves were taken and the actual band widths at 0.6064 $d_{obsd.}$ were measured. Both the 0.15- and 0.30-mm. slit width curves for n-1-decene gave 0.69 as the mean of four values for the constant (in density times wave numbers per 1%). This implies 6.2 cm.$^{-1}$ for the true band width, $2l$. Similarly, the two runs at 0.17-mm. and 0.26-mm. slit width for p-diisopropyl benzene gave 2.8 and 2.9 cm.$^{-1}$ band width, respectively. The lower value from the narrower slit width data is preferred. Values of 9.6, 11.1, and 12.7 cm.$^{-1}$ were given for the band width of cetane by the results at 0.265, 0.80, and 1.30 mm. Extrapolation to zero slit width gives 9.5 cm.$^{-1}$

Multiplying these values of the band width by the values of n gives the "equivalent slit width" of the spectrometer (by definition). A comparison of these values with the "effective slit width" (calculated as the sum of the geometrical slit width and diffraction function from data supplied by the manufacturers of the spectrometer) is given in Table III.

Although no simple relationship was expected, it can be seen that the equivalent slit width is roughly the effective slit width plus a constant. It should be remembered that the low value in cetane is very approximate and also that the cetane band shows some signs of asymmetry at high resolving power. It is clear, however, that the differences in Table III are not real physical quantities, inasmuch as they do not vary systematically with wave length or band width. In addition, they are too large to be actual aberration of the final image in the spectrometer. The instrument has resolving power up to the maker's specifications, so that an aberration of 0.4 mm. ($\approx$ 4 cm.$^{-1}$) at 913 cm.$^{-1}$ is scarcely feasible.

In view of the approximations involved in the original premises, it was unlikely that the parameters deduced would have a real physical significance. Nevertheless if an experimental relationship is established between effective and equivalent slit widths (or more practically, between n and slit width in millimeters), results at different slit widths can be correlated for a particular absorption band.

CONCLUSION

The effect of finite slit width even with instruments of reasonably high resolving power is to cause large deviations from Beer's law. Barnes (*1*) states that organic molecules have absorption bands of the same order of magnitude as the spectral slit widths (his value of 10 to 15 cm.$^{-1}$ is probably large). The condition that they are equal implies an error of 15% from the true value of the calibration coefficient, and (more important) a variation of 3% in calibration coefficient for 10% change in slit width. Because so many infrared spectrometers use the slit width as a variable in obtaining an incident intensity independent of wave length, care must be taken to ensure that the slit width used for a series of measurements is constant.

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Quantitative Analysis with Infrared Spectrophotometers

DAVID Z. ROBINSON
Baird Associates, Inc., Cambridge 38, Mass.

This work was started as part of a program of evaluation of infrared spectrophotometers for quantitative analysis. It is shown that in addition to errors in transmission, there are errors due to incorrect reading of the 0 and 100% lines. Deviations from Beer's law are to be expected whenever the slits are wider than the width of the absorption band used. The law of additivity of optical density will not hold if Beer's law does not hold. Double- and single-beam infrared spectrophotometers are compared for use in quantitative analysis. The possibility of making direct determination of the true 100% line and of eliminating the spectra of one or more of the components of a mixture are two special features of the double-beam spectrophotometer.

THE purpose of this paper is to give a systematic outline of some errors in infrared spectrophotometric analysis. The explanations deal with double-beam instruments, as well as single-beam spectrometers.

A number of authors have described quantitative methods in infrared spectroscopy. Wright (*5*), Barnes *et al.* (*1*), Heigl *et al.* (*3*), and Brattain *et al.* (*2*) have mentioned some of the errors involved and have described many techniques. Some of their results are described in this paper for the sake of completeness, but the sections calculating Beer's law deviations and some of the calculations of errors are believed to be new.

Chemical analysis by the measurement of infrared transmittance is in essence not very different from ordinary colorimetry. Some special problems occur, such as the narrow absorption bands, the existence of stray light, and the presence of atmospheric absorption which lead to small but important differences.

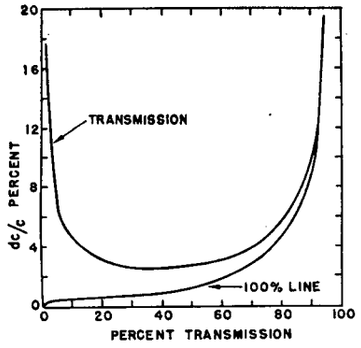

Figure 1. Per Cent Error in Concentration

For 1% error in transmittance (*upper*) or 1% error in 100% line (*lower*)

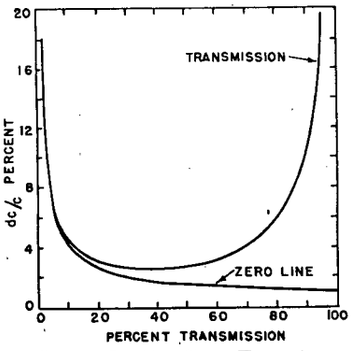

Figure 2. Per Cent Error in Concentration

With 1% error in concentration with 1% error in transmittance (*upper*) or 1% error in zero line (*lower*)

There are two main types of infrared spectrometers. The single-beam spectrometers measure the energy of the incoming beam as a function of wave length. Such instruments give an absorption spectrum which is superimposed on the curve of source intensity and atmospheric absorption. In some cases the source intensity changes are approximately compensated by automatic adjustment of the slit opening. Double-beam spectrophotometers compare the light energy going through the sample with the energy going through a reference cell. Such systems eliminate the atmospheric bands and the source changes. In principle, a single-beam spectrometer plus computation gives results equivalent to those from a double-beam spectrophotometer, with the exception that the reference curve has been run at a different time. In practice there are many convenient advantages of double-beam instruments.

BEER'S LAW

All measurements of concentration are based on the fact that the transmittance of light through a cell containing a number of molecules is a function of the number of molecules in the light beam. If the light is monochromatic, or approximately so, the simplest approximation to this function is the Beer-Lambert law. If we are considering a solution of one component in a nonabsorbing solvent, then Beer's law in a slightly changed form can be expressed by Equation 1

$$c = \frac{-1}{a(\lambda)\, d} \ln t(\lambda) \qquad (1)$$

where c is the concentration of the component, $a(\lambda)$ is the absorptivity at this wave length, d is the thickness, and $t(\lambda)$ is the transmittance of the sample at the wave length in question. For most analytical measurements the transmittance minimum of a band is used as the measure of its concentration if the band is isolated. Every measurement of transmittance involves three readings: the energy transmitted at the minimum, the true 100% line—i.e., the line of no absorption—and the true zero line (accounting for stray light). The effect of errors in each of these on the errors in concentration is discussed separately.

ERROR IN MEASUREMENT OF TRANSMITTANCE

Because the limiting factor in making transmittance measurements is usually noise, which is independent of the amount of energy hitting the receiver, if we assume that the error in transmittance, dt, is a constant independent of t, it is easy to predict the effect of such an error (see, for example, *1*).

By differentiating the logarithm of Equation 1, Equation 2 is obtained:

$$\frac{1}{c}\, dc = \frac{dt}{t \ln t} \qquad (2)$$

If we now assume that dt is a constant, say 1%, then the error in concentration plotted as per cent of amount present can be computed as a function of concentration. This error in transmittance is the top curve of Figures 1 and 2.

If all the error in concentration were due to a constant error in transmittance, the minimum error would be made when the thickness is so adjusted that the transmittance is $1/e$ or 37%. Actually, any transmittance between 20 and 60% would not lead to a greatly different error. This result is, of course, well known and is derived merely for comparison with the results that follow.

ERROR IN MEASUREMENT OF 100% LINE

When absorption is being measured, it is necessary to measure the original intensity and to make proper allowance for the reflection and scattering losses. In a double-beam instrument the latter two can be compensated by putting equivalent losses in the reference beam. Very often a thick plate of rock salt is adequate. The 100% line is then adjusted with an opaque shutter in one beam.

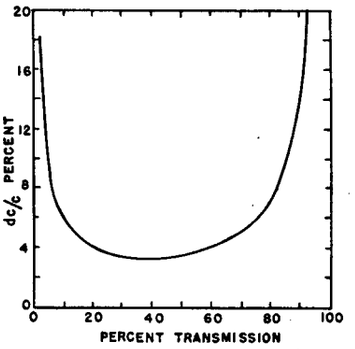

Figure 3. Probable Per Cent Error in Concentration

With 1% error in transmittance, 100% line and zero line

In a single-beam instrument the "base-line" method is often used. Instead of a true 100% line, a straight line is drawn between two transmittance maxima on each side of the band. The height of this line at the transmittance minimum is used as the 100% line. A more precise method uses a dummy cell, just as in the double-beam system.

In both these methods, an error in the setting of the 100% line is as easy to make as an error in transmittance. It is possible to calculate the effect of this error in the following way:

Because the relationship between c and t has already been obtained, it is only necessary to get the relationship between t and I_0.

$$t = I/I_0 \tag{3}$$

$$\frac{dt}{t} = \frac{-d\,I_0}{I_0} \tag{4}$$

By substituting Equation 4 in Equation 2, Equation 5 is obtained.

$$\frac{dc}{c} = \frac{-1}{\ln t}\,\frac{d\,I_0}{I_0} \tag{5}$$

If the assumption that $d\,I_0/I_0 = 1\%$ is made, the effect of this error in the 100% line on concentration can be calculated. The bottom curve in Figure 1 shows the per cent error in concentration with a 1% error in the 100% line as a function of transmittance.

As might be expected, the error is small at low transmittance, and approaches the error due to a transmittance error at high transmittance. Because this measurement is as likely to give error as a transmittance measurement, it would seem more advantageous to work at slightly lower transmittance than 37%.

ERROR IN ZERO LINE

Every measurement of transmittance requires a determination of the zero line. This is not usually the point where an object opaque to all wave lengths transmits, but is ideally the energy transmitted by an object which is perfectly transparent at all wave lengths except that where the measurement is being made.

In practice, it is impossible to determine the stray light in this manner. Instead, a material that is transparent in the short wave-length region and opaque at the spectrometer setting is put into the beam. This only gives a close lower limit to the stray light, inasmuch as some of the short wave-length radiation is reflected and all the longer wave-length stray light is absorbed.

By putting a large amount of the material to be measured in the beam, it will usually absorb completely in the region desired. However, these thick samples will absorb some of the short wave-length stray light. Often no correction for stray light is made, other than its removal by a filter of some kind. Any of the above methods gives a good approximation to the correct determination of the zero line.

The error in composition due to an error in the zero line can be determined by determining the effect on the transmittance and thus on the concentration.

Stray light is corrected for in practice by subtracting the amount of stray light from both I and I_0 (Equations 1 and 2)

$$t = \frac{I - S}{I_0 - S} \tag{6}$$

where I and I_0 have been observed on the chart

$$\frac{dt}{t} = \left[\frac{1}{I - S} - \frac{1}{I_0 - S}\right] dS \tag{7}$$

If we now assume that the stray light has been estimated as being zero, the expression can be simplified.

$$\frac{dt}{t} = \frac{1 - t}{t}\,\frac{dS}{I_0} \tag{8}$$

By substituting Equation 8 in Equation 2, Equation 9 is obtained.

$$\frac{dc}{c} = \frac{1 - t}{t \ln t}\,\frac{dS}{I_0} \tag{9}$$

If the assumption is made that $dS/I_0 = 1\%$, then dc/c can be computed. The per cent error in concentration for a 1% error in the zero line is given by the bottom curve in Figure 2.

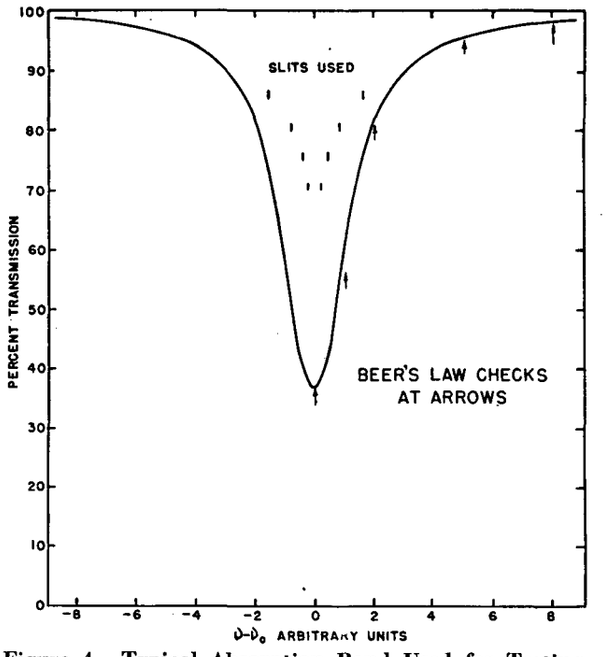

Figure 4. Typical Absorption Band Used for Testing Beer's Law

Showing positions of test (arrows) and slit widths used

The error due to a wrong zero is least (but not zero) at high transmittance and approaches the error due to a 1% transmittance error as we approach low transmittance.

For best results the point of minimum error is probably not 37% but, instead, differs from instrument to instrument, even when Beer's law holds. Figure 3 shows a plot of the per cent error in concentration with a probable error of 1% in zero line, 100% line, and transmittance. The minimum occurs at about 40% transmittance, but any transmittance between 25 and 60% gives about the same error.

DEVIATIONS FROM BEER'S LAW

Beer's law is simply a statement that equal path lengths of absorber will absorb equal fractions of light. This statement seems so obvious that it is difficult to see why it should not hold as long as there is no unusual molecular interaction. Beer's law, however, does assume that the light coming through the cell is monochromatic. This is, of course, only approximately true. All spectrometers send a small bundle of wave lengths centered around the wave-length setting of the instrument. If the slits are so wide, or the absorption bands so narrow, that there are rapid changes in absorption coefficient over a slit width, deviations will occur.

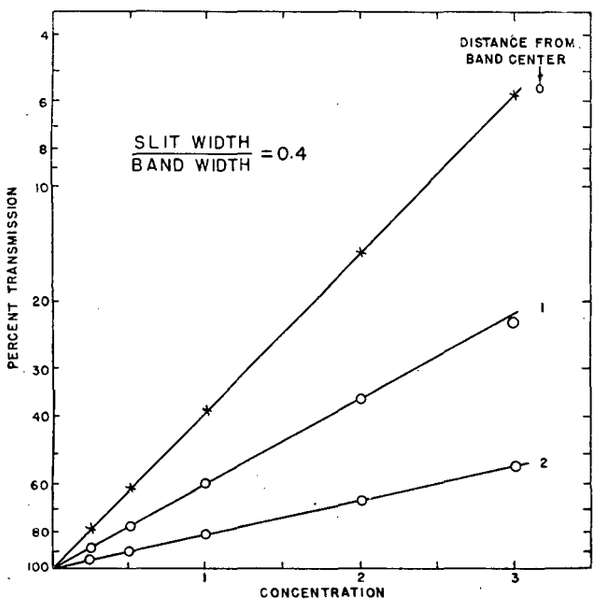

Figure 5. Apparent Log t *vs.* Concentration for Slit 0.8 Unit Wide

If Beer's law held, straight line would go through all points. Distance from "band center" refers to points marked on Figure 4

It is possible to calculate the magnitude of the deviations if a simplifying assumption is made. It is assumed that the spectrometer averages the transmittance over a slit width. This assumption is not quite correct, because most instruments do not have square slit functions and therefore take a weighted average of the transmittance. If a weighting function is used to average the transmittance, the calculations become much more difficult and the results are but slightly changed.

Consider the typical band plotted in Figure 4, where the absorption coefficient follows Equation 10.

$$a = \frac{A}{1 + \nu^2} \tag{10}$$

Here A is proportional to the concentration and the width at half height is arbitrarily defined as 2 units.

The positions tested for deviations are shown with arrows on Figure 4. They are at the band minimum (0), at the point where the absorption coefficient is one half that of the maximum (1) and 2, 5, and 8 units away from the minimum.

For testing Beer's law it is necessary to use a number of slit widths, each at a number of different concentrations. The slit widths chosen had widths 0.2, 0.4, 0.8, and 1.6 times the band width and the concentration range used was such as to make the true transmittance at the band minimum go from 5 to 95%.

The calculations were made by averaging the transmittance (not the absorption coefficient) at the different band positions over various slit widths at these various concentrations. The results are summarized in Figures 5 through 7. In these curves the logarithm of the calculated transmittance is plotted against the concentration for the various band positions. If Beer's law held, all these lines would be straight. When the slit width is 0.4 unit wide ($^1/_5$ the band width) there are no appreciable deviations.

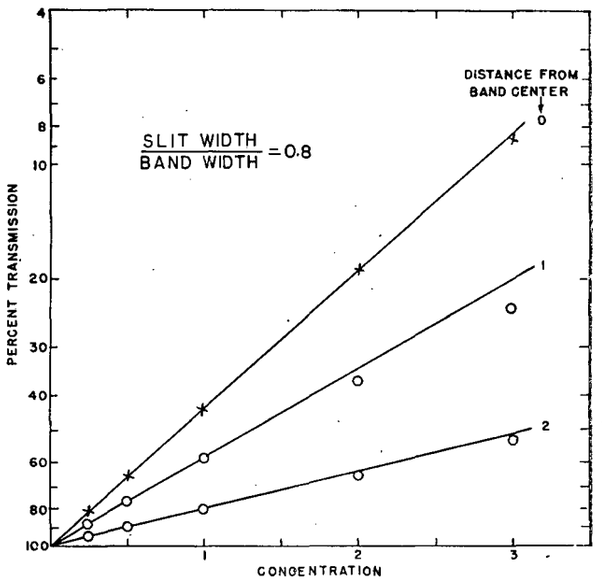

Figure 6. Apparent Log t *vs.* Concentration for Slit 1.6 Units Wide

Deviations from straight line are occurring. Symbols same as in Figure 5

With a slit $^2/_5$ the band width, deviations appear at the point where the absorption coefficient is varying widely, the half-height point 1 unit away from the maximum. Although Beer's law still holds for the band minimum and far from the minimum, it does not hold for the side of the band (Figure 5).

With a slit $^4/_5$ the band width (Figure 6) deviations occur at the band minimum and 2 units away from it, but the greatest deviations still occur at the side of the band. Figure 7 shows the results for a slit appreciably wider than the band. For low transmittance, large deviations are present for 0, 1, and 2 units away from the band minimum.

For all these slits no deviations occurred when Beer's law was tested 5 and 8 units away from the minimum. This result was not surprising, because there were only slight variations in absorption coefficient over the slit. Furthermore, all the curves approached a straight line as the concentration decreased.

As a general conclusion it can be stated that as long as the slit width is less than the width of the band being studied, and the transmittance is in the usual range of study, there should be no deviations from Beer's law. If, however, the slit widths are appreciably wider than the band widths, deviations will occur at lower transmittances.

EFFECT OF BEER'S LAW DEVIATIONS ON ACCURACY

Because it is always possible to draw a working curve, plotting the transmittance as a function of concentration, how should Beer's law deviations affect the results? From Figures 6 and 7 it

may be seen that a 1% change in transmittance will lead to a greater error in concentration whe deviations occur.

Because the deviations occur at lower transmittances, one might have to work in the transmittance range above 40% for the most accurate results.

LAW OF ADDITIVITY OF OPTICAL DENSITY

The law of additivity of optical density states that the optical density (log I_0/I) of a mixture is equal to the sum of the optical densities of its components. This means that equal absorbing paths of the same material will always absorb the same fraction of the incident light. The law of additivity of optical density is just a corollary of Beer's law.

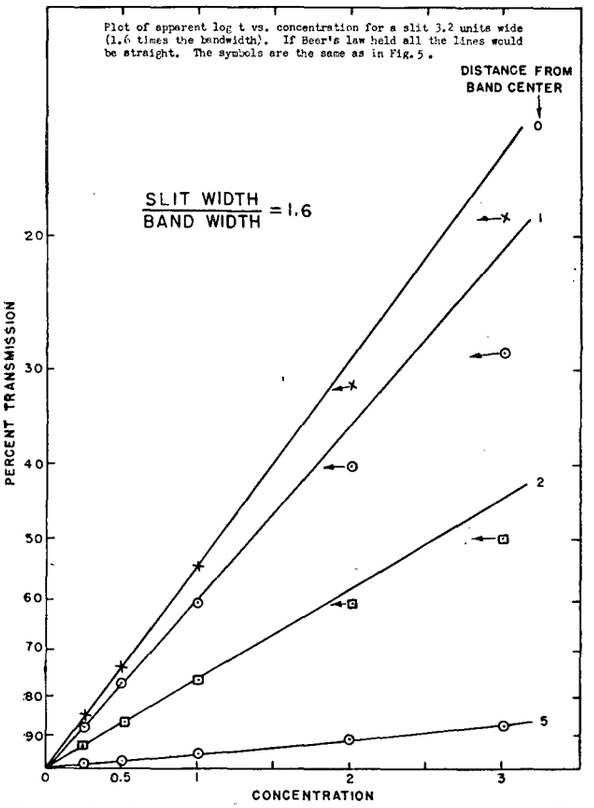

Figure 7. Apparent Log *t* vs. Concentration for Slit 3.2 Units Wide

Some authors have made the statement that where Beer's law did not hold, the law of additivity of optical densities would still hold true. It is of interest to examine the cases where this statement holds. If Beer's law holds for both components, then the law of additivity of optical density would obviously be expected to hold. Even if Beer's law held for one component, the additivity law might still hold. This can be seen most easily if we consider the two components separately. After the light has gone through the first component, the one obeying Beer's law, its spectral distribution has not been changed. Therefore, the second component will act just as if the first one was not there, so far as the fraction of light that it would remove was concerned.

If, however, neither component obeyed Beer's law at the wave length observed, there would be no reason for the mixture to obey the law of additivity of optical density. In fact, if we were to shine the light through each component separately, the light going through the first component would have its spectral distribution radically changed. Now when this light goes through the second component the fraction removed would not be the same.

The simplest example is that where both components have the same band. Consider the case described by the top curve of Figure 7 with the band minimum. The computed results show that the optical density for a concentration of 1 is 0.263, whereas for a concentration of 2 it is 0.484. The optical density of the sum of two cells each with a concentration of 1 would be a good deal greater than the actual density observed. This direction of error is the one expected. Even though the term absorption coefficient is eliminated, the deviations are not.

There can be cases where Beer's law works for the band minimum but not the side of the band (Figure 5). Then when we are trying to detect concentrations by observing a shoulder on the side of the band, the law of additivity of optical density would not hold, even though Beer's law held for the band minimum.

For the additivity law to hold, Beer's law must hold for all but one of the components, at the wave lengths being studied.

USE OF COMPONENT CANCELLATION IN QUANTITATIVE ANALYSIS

One of the advantages of a double-beam spectrophotometer is that it enables the operator to eliminate the spectrum of one or more of the components, by putting the same amount of the component in the reference beam.

If a multicomponent mixture is to be analyzed, then in principle it is possible to cancel out the spectra of each component successively. This procedure enables the analysis to be made without overlapping bands. Such a procedure is too long for most purposes, but is very useful when small amounts of impurities are to be determined.

To check this experimentally, some spectra were taken with the Baird Associates, Inc., double-beam spectrometer. The bottom half of the chart in Figure 8 shows a spectrum of a crude *p*-cresol sample dissolved in carbon disulfide. The only difference between its spectrum and the spectrum of a refined *p*-cresol sample was the shoulder on the 13.5μ band.

For any kind of quantitative analysis it would be obviously difficult to determine the difference in optical densities between the shoulder on the side of the band. By canceling out the spectrum of the pure material it would be easy to determine the impurity.

To do the cancellation most simply it would be best if the amount of pure material were the same in each cell. If the two cells differed in thickness, the concentration of the mixtures should be different.

The thickness of a cell can be determined by observing the spectrum of an empty cell. Because of interference between the beam going through the cell and the beam reflected off the inside

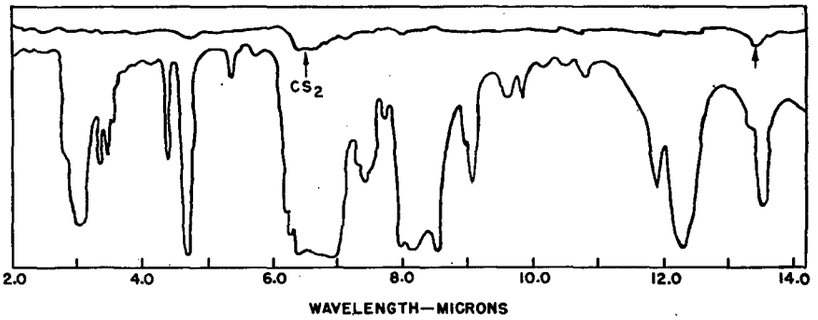

Figure 8. Double-Beam Analysis Using Compensation

Lower curve shows spectrum of crude *p*-cresol sample dissolved in CS_2. Upper curve is spectrum of same sample with same amount of pure *p*-cresol in reference beam.

of the cell, the spectrum consists of transmittance maxima and minima (*4*). (It is very difficult to obtain the true position of the maxima and minima with a single-beam spectrophotometer because of the change in source energy with wave length.) The thickness of the cells differed by about 15%. The carbon disulfide solution of the crude material was made 15% weaker than the solution of the pure *p*-cresol.

The solution of crude *p*-cresol was placed in the longer cell and the cell was run against the stronger solution of pure material placed in the reference beam. The differential spectrum is shown in the upper part of the chart in Figure 8. The 13.5μ band is clear and its area or height can be used for quantitative work. The dip at 6.5μ is due to the extra carbon disulfide in the sample beam. The instrument can follow the ratio of the curves until both beams absorb essentially all the light coming through. There is no change in the deviations from Beer's law when one of the bands is canceled out, because the spectral distribution of light is still uneven.

SUMMARY OF RESULTS

It is important with either double- or single beam-spectrometers to take account of the stray light and the true 100% line. Errors due to apparent deviations from Beer's law should occur whenever the slit width is wider than the width of the band being studied. The law of additivity of optical density will not work when Beer's law does not work for all the components in a mixture.

Double-beam spectrophotometers have a number of conveniences for quantitative work. The use of compensation techniques to get rid of component spectra and the easy way to obtain a good 100% line are among the advantages.

ACKNOWLEDGMENT

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Infrared Absorption Spectra of Some Epoxy Compounds

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

In speculating on the mechanisms of the autoxidation of unsaturated compounds, some investigators have postulated that various kinds of heterocyclic oxygen ring compounds are formed. There is no reliable evidence, however, of the formation of any type of oxygen ring compounds except oxirane compounds. Infrared absorption spectra of a series of pure epoxy compounds from 2 to 15 microns have been obtained and interpreted. Oxirane derivatives of terminally monounsaturated compounds show two characteristic absorption bands near 11 and 12 microns; oxirane derivatives of cis monounsaturated fatty acids, esters, and alcohols show a characteristic band at 11.8 to 12 microns, whereas those from the trans isomers show a band at 11.2 to 11.4 microns; absorption bands characteristic of 5- and 6-membered carbon-oxygen rings have been noted. The spectra of the long-chain epoxy acids and alcohols are dependent on physical state. The spectra are primarily intended to serve as reference data in the application of infrared spectrophotometric methods to the analyses of autoxidation mixtures.

IN SPECULATING on the mechanism of the reaction of unsaturated compounds with oxygen, some investigators have postulated that various kinds of heterocyclic oxygen ring compounds are formed. There is no reliable evidence of any type of heterocyclic oxygen ring compound except oxirane compounds, which have been isolated from such reactions. Even when oxirane compounds are not isolated, they can be determined quantitatively in systems involving oxidation of monounsaturated compounds (*11*). In the oxidation of polyunsaturated compounds, however, conjugated materials, which form early in the reaction, interfere with the analytical determination of oxirane compounds. The authors know of no specific analytical method for detection or quantitative determination of other types of ring oxygen, especially when present in small quantities and with other functional groups.

For the qualitative (*3*), as well as the quantitative or semiquantitative (*1*), determination of functional groups, infrared spectroscopy has recently become a useful tool. Little has been reported, however, on the spectra of heterocyclic oxygen compounds. The few published spectra (*2*) cover only a limited range, and the purity of the compounds is unknown.

Since this paper was submitted, Field, Cole, and Woodford (*4*) reported infrared absorption data on eight oxirane compounds. They concluded that only the 1250 cm.$^{-1}$ (8μ) band could be identified with reasonable certainty as characteristic of the oxirane group.

Before infrared spectroscopy can be used to detect the presence or absence of heterocyclic oxygen ring compounds in oxidation reaction mixtures, and in other applications, it is essential to have reference spectra of pure compounds. This paper, which reports an extension of earlier work (*7*), gives the infrared absorption spectra from 2 to 15 microns of thirteen pure oxirane compounds, tetrahydropyran, tetrahydrofuran, and dioxane. The spectra are interpreted in the discussion.

EXPERIMENTAL

Spectrophotometer. The spectrophotometer and techniques used were identical with those described in a previous paper

Table I. Characteristics of Reference Compounds

Reference Compound	B.P. ° C.	B.P. Mm.	M.P. ° C.	n_D	Oxirane Oxygen, % (11)	Method of Preparation and/or Literature Reference
Glycidol	39.3	2.5	...	1.4293 (25°)	21.3	(9)
Propylene oxide	33.5	...	...	1.3670 (20°)	...	Distillation of best commercial grade through efficient column (50 to 120 plates)
3,4-Epoxy-1-butene	66	...	...	1.4176 (20°)	...	
1,4-Dioxane	101	...	...	1.4223 (20°)	...	
Tetrahydrofuran	66	...	...	1.4074 (20°)	...	
Tetrahydropyran	88	...	...	1.4210 (20°)	...	
1-Phenyl-1,2-epoxyethane (styrene oxide)	100	40	...	1.5295 (30°)	...	
1,2-Epoxydecane	87.6	10	...	1.4249 (30°)	9.81	(10)
1,2-Epoxydodecane	98-99	3.5	...	1.4314 (30°)	8.45	(10)
1,2-Epoxytetradecane	101.5-102.4	0.5	...	1.4363 (30°)	7.23	(10)
cis-9,10-Epoxystearic acid	...	...	59.8	...	5.38	(5)
trans-9,10-Epoxystearic acid	...	...	55.6	...	5.37	(5)
Methyl *cis*-9,10-epoxystearate	120-128	0.015	...	1.4479 (30°)	5.10	(5)
Methyl *trans*-9,10 epoxystearate	126-128	0.018	...	1.4449 (30°)	5.02	(5)
cis-9,10-Epoxyoctadecanol	...	...	53	...	5.60	(5)
trans-9,10-Epoxyoctadecanol	...	...	48.3-48.7	...	5.64	(5)
n-Decane	...	...	...	...	...	Natl. Bur. of Standards sample
Thermally treated *cis*-9,10-epoxystearic acid	...	...	...	...	...	*cis* - 9,10 - Epoxystearic acid, m.p. 59.8°, heated 26 hours at 100° (8)

(7). The spectra of the liquids were determined in a 0.033-mm. liquid cell, except the spectrum of glycidol, which was determined in a 0.015-mm. cell. The spectra of the solid compounds were determined on 10% solutions in carbon bisulfide in a 0.115-mm. cell and also on the solid as Nujol mulls.

Materials Used. The reference compounds employed, some of their characteristics, and methods of preparation are shown in Table I.

RESULTS AND DISCUSSION

Figures 1 to 6 show spectra for the various compounds as plots of per cent transmission against wave length in microns on a linear wave-length scale. Wave-length positions of absorption maxima, read from the original records, are shown on each curve.

Oxirane Derivatives of Long-Chain Olefins. To determine the effect of introducing the oxirane ring into a long-chain compound, spectra of the oxirane derivatives of three terminally unsaturated long-chain olefins (1-decene, 1-dodecene, and 1-tetradecene) were obtained. In Figure 1 these spectra are compared with that of a standard National Bureau of Standards sample of *n*-decane at the same cell thickness. Bands of medium intensity near 7.0, 7.9, and 8.8, as well as two strong bands near 10.9 and 11.9 microns, are present in the spectra of the three oxirane derivatives but absent in that of the hydrocarbon. 1-Dodecene and 1-tetradecene, like the saturated hydrocarbon, do not absorb appreciably at these wave lengths at comparable sample thickness. Probably the first three of these bands are caused by various C—O vibration modes, and the two strong bands near 10.9 and 11.9 microns are caused by motions of the oxirane ring, which vibrates as a unit.

Oxirane Compounds of Lower Molecular Weight. Figure 2 shows spectra of four additional compounds that contain the oxirane ring in the terminal position. The origin of many of the bands can be deduced from the molecular structure, in conjunction with well-known frequency assignments. All four compounds show strong bands in the vicinity of those observed near 11 and 12 microns in the long-chain oxirane compounds of Figure 1. Propylene oxide and glycidol show these bands altered in relative intensity but at nearly the same wave lengths as do the compounds of Figure 1. In the spectrum of 3,4-epoxy-1-butene, however, the longer wave-length band of this pair has undergone a considerable shift, probably as a result of the effect of the adjacent double bond on the vibration involved. Ambiguity arises in the spectrum of 1-phenyl-1,2-epoxyethane (styrene monoxide) because of the possibility that strong bands in this region are caused by the monosubstituted aromatic ring. (Styrene, for example, has a strong band at 11 microns.)

Oxirane Derivatives of Long-Chain 9-Monounsaturated Acids, Esters, and Alcohols. From the foregoing discussion, it may be tentatively concluded that an oxirane ring in the terminal position in a molecule causes a pair of absorption bands near 11 and 12 microns. Figure 3 shows spectra of the oxirane derivatives of oleic acid, methyl oleate, and oleyl alcohol; also shown are

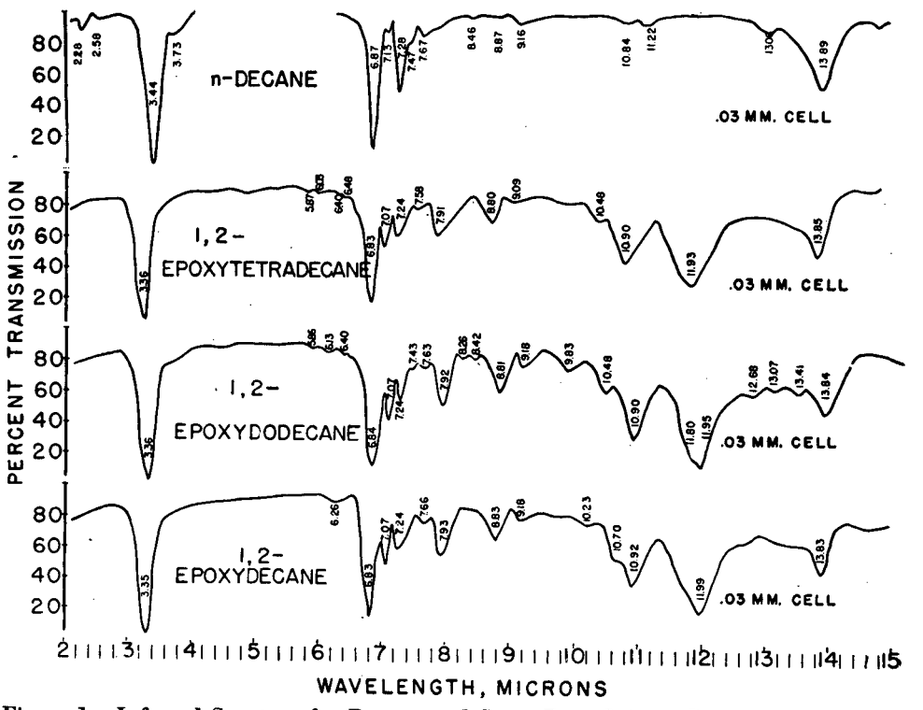

Figure 1. Infrared Spectra of *n*-Decane and Some Pure Oxirane Derivatives of Long-Chain 1-Olefins

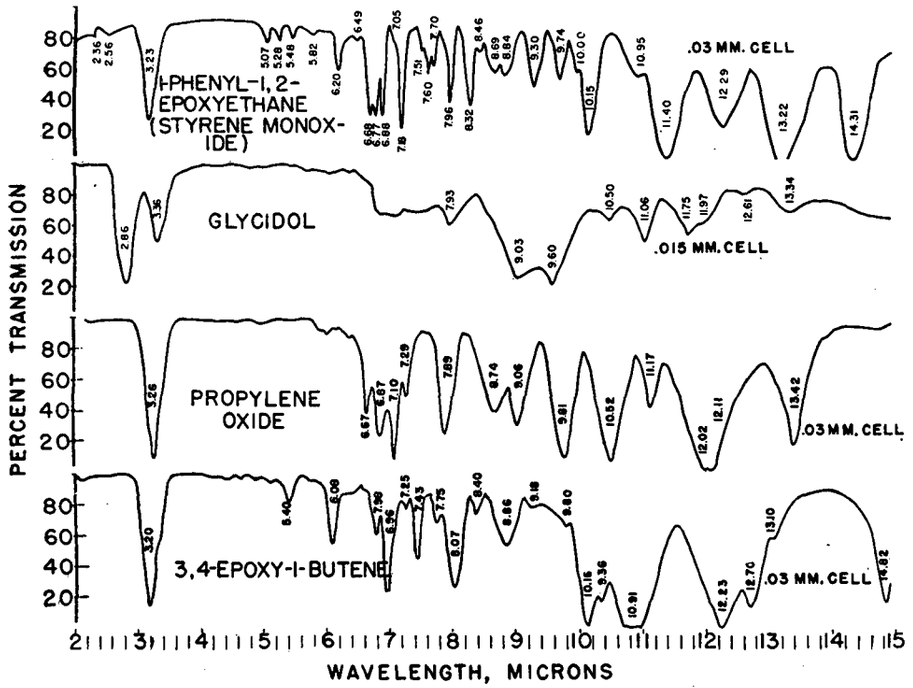

Figure 2. Infrared Spectra of Some Pure Oxirane Compounds of Lower Molecular Weight

the spectra of the oxirane derivatives of the trans isomers of these three compounds (elaidic acid, methyl elaidate, and elaidyl alcohol). The effect of introducing the oxirane group into these long-chain acids, esters, and alcohols is best illustrated by comparing (*a*) the spectrum of each epoxy acid with that of stearic acid, (*b*) the spectrum of each epoxy ester with that of methyl stearate, and (*c*) the spectrum of each epoxy alcohol with that of stearyl alcohol (*n*-octadecanol). The three spectra required for this comparison were discussed in a previous paper (*6*). Such a comparison shows that the spectra of the *cis*-epoxy acid, ester, and alcohol are qualitatively similar to those of stearic acid, methyl stearate, and *n*-octadecanol, respectively, except for a new band near 12 microns in each case. Similarly, the spectra of the *trans*-epoxy acid, ester, and alcohol show close qualitative similarity to those of stearic acid, methyl stearate, and *n*-octadecanol, respectively, except for a new band near 11.2 microns. The 11.2-micron band in each of the trans oxirane compounds is somewhat stronger relative to other bands in the spectrum than is the 12-micron band in the corresponding cis compound. By analogy with the changes caused by introducing the oxirane ring into the long-chain hydrocarbons, these changes in the 7-, 7.9-, and 8.8-micron region were expected. Because C—O linkages are already present in the comparison compounds, however, such changes are not readily apparent.

On the basis of these observations, together with those noted in connection with the spectra of Figures 1 and 2, the authors have tentatively concluded that the oxirane ring in oxirane compounds derived from long-chain internally monounsaturated compounds having the cis configuration at the double bond causes an absorption band near 12 microns. This ring in the corresponding trans compounds causes a band near 11.2 microns. If the oxirane compound is derived from a terminally unsaturated compound, bands appear near both these wave lengths.

Effect of Physical State on Spectra of Solid Long-Chain Oxirane Compounds. The spectra shown in Figure 3 for the solid oxirane compounds (the epoxy acids and alcohols) were determined on solutions of these compounds in carbon bisulfide. To determine their spectra in the solid state, these four compounds were also used as finely divided powders dispersed in Nujol (Figure 4). Although some minor differences were expected, the marked changes observed were surprising.

Comparison of Spectra of Epoxy Acids in the Solid State and in Solution. In a previous paper (*7*) it was pointed out that the long-chain fatty acids show several common spectral features, with reference to both position and general pattern of absorption maxima. As implied in the comparison with stearic acid above, the spectra of solutions of the two epoxy acids (Figure 3) exhibit all these typical spectral features, and therefore show a general resemblance to the spectra of unmodified fatty acids. In the spectra of solids (Figure 4), however, several of these typical absorption patterns have undergone drastic alteration. The broad absorption which appears in the 8-micron region in the spectra of long-chain acids (*7*) is normal in the spectra of the epoxy acids in solution (Figure 3), but is replaced in the spectra of the solids by a series of sharp, well-resolved bands. These are intense in the *cis*-epoxy acid but weak in the *trans*-epoxy acid. The broad band near 10.7 microns, typical of acids (*7*), is normal in the spectra of solutions; in the spectrum of the *cis*-epoxy acid in the solid state, the strong 10.9-micron band probably corresponds to this absorption. If so, a substantial shift in wave length, together with a sharpening and increase in intensity (relative to other bands) has occurred. In the *trans*-epoxy acid, the weak 11.1-micron band evidently corresponds to this absorption, indicating an even greater shift and a decrease rather than an increase in intensity. The 13.9-micron band, related to CH_2 rocking motions in the long-chain acids (*7*), is also normal in the solution spectra of the epoxy acids. In the spectra of solids, the absorption of Nujol near this wave length obscures the issue; it is apparent, however, that this band in the spectrum of the *cis*-epoxy compound is either shifted to a shorter wave length or has been resolved into a doublet, probably the latter. In the spectrum of the *trans*-epoxy acid, this absorption appears as a single band in the spectra of both solution and solid. The weak band near 3.7 microns, believed to be a branch of the O—H . . . O "association" band in the spectra of fatty acids (*7*), appears to be normal in the spectra of solutions but is absent in the spectrum of the solid *trans*-epoxy acid and nearly absent in that of the *cis*-epoxy acid.

In addition to the alterations in the various absorption maxima which are typical of long-chain fatty acids, the 12-micron band attributed to the oxirane ring in the spectrum of the solution of the *cis*-epoxy acid has shifted to 11.8 microns in the spectrum of the solid and is greatly increased in sharpness and intensity. A similar increase, together with a shift from 11.2 to about 11.4 microns, has occurred in the oxirane ring band in the spectrum of the *trans*-epoxy acid. In general, the transition from solution to solid seems to be more marked for the *cis*- than for the *trans*-epoxy acid.

Comparison of Spectra of Epoxy Alcohols in the Solid State and in Solution. Although the spectra of solution of the two epoxy alcohols (Figure 3) show all the features common to long-

chain alcohols, the following changes are apparent in spectra of the solids (Figure 4): As expected, the hydroxyl stretching band near 2.8 microns in the spectra of solutions shifts to about 3.0 microns as a result of increased hydrogen bonding; the hydroxyl bending absorption near 9.5 microns, common to long-chain alcohols, shifts to about 9.3 microns; and the CH_2 rocking absorption at 13.9 microns, as in the spectrum of the *cis*-epoxy acid, is apparently resolved into a doublet.

In addition to these changes in the typical long-chain alcohol absorption bands, a number of new bands, entirely absent in spectra of the solutions, appear throughout spectra of the solids beyond about 8 microns as in the epoxy acids. The cis oxirane ring band has shifted from 12 to about 11.8 microns, the trans oxirane band has shifted from 11.2 to about 11.4 microns, and both bands are sharper and more intense.

Although some of the marked effects of physical state on the spectra of the solid epoxy compounds can be attributed to formation or breaking of hydrogen bonds or to dipolar association, many of the differences cannot be explained on this basis A similar phenomenon has been observed by Richards and Thompson (*6*) in spectra of nonpolar hydrocarbons, compared in the solid and molten state. These workers found that the effect was more pronounced with long-chain paraffins than with "more rigid structures" such as the alkyl-substituted benzenes. As in the present case, many broad bands in the spectra of liquids split into two or more bands in the spectra of solids and, in general, bands in the spectra of solids were more numerous and sharper. As suggested by Richards and Thompson, passage from the liquid (or solution) to the solid state may involve changes in the potential energy functions associated with molecular vibration, and alteration in molecular symmetry and ordered arrangement may result in changes in the selection rules and principles governing the infrared activity of vibrations.

As noted above, the absorption band attributed to a vibration of the oxirane ring appears broad and weak in the spectra of solutions but sharp and strong in those of solids. This change may be related, at least in part, to the fact that in the ordered crystal state all individual groups of a given type probably vibrate in nearly identical field environments, and therefore absorb at nearly identical frequencies. The total absorption for all such groups would be concentrated over a narrow frequency range, producing a strong sharp band. In the unordered solution (or molten) state, however, variations in the environment of the individual groups would cause variations in exact positions of absorption maxima, with consequent broadening and decrease of over-all intensity of the observed band.

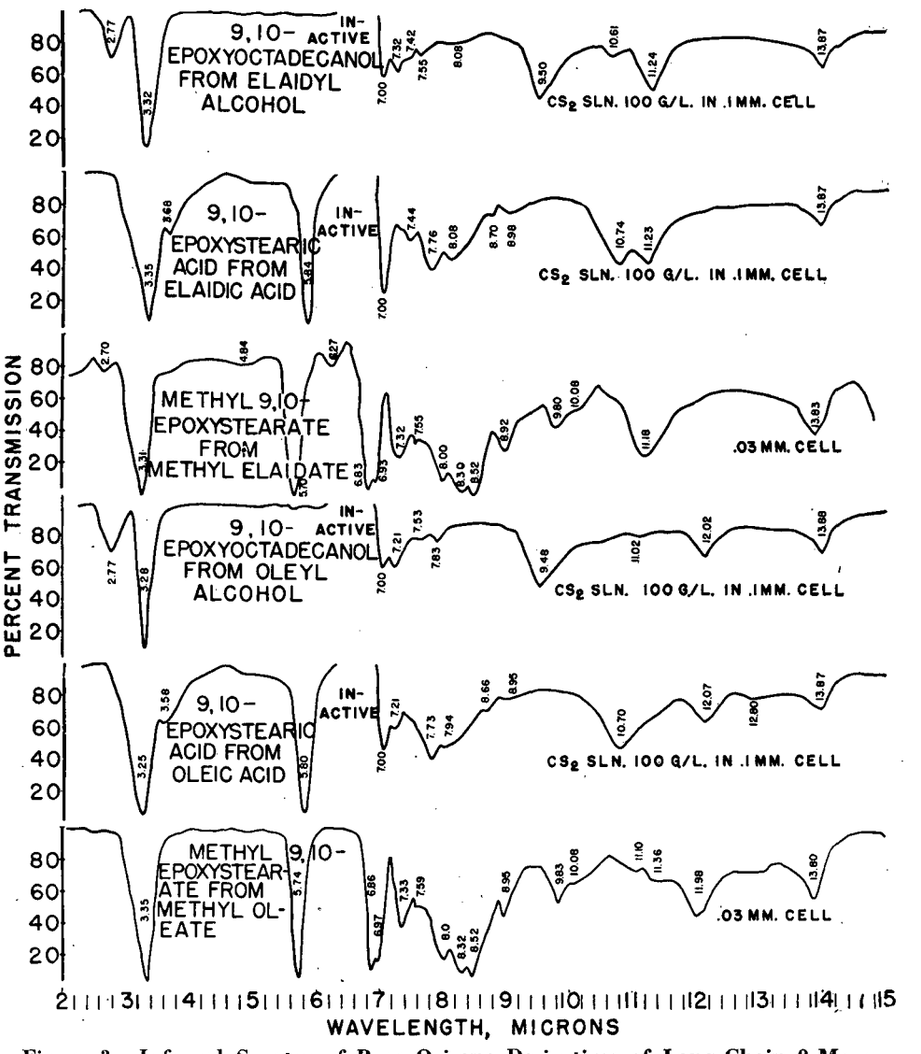

Figure 3. Infrared Spectra of Pure Oxirane Derivatives of Long-Chain 9-Monounsaturated Acids, Esters, and Alcohols

Differences between spectra of solids and solutions are usually minor, as compared with those shown here, and the more marked differences are usually attributable to differences in degree of association through polar groups. The effect observed in this work, however, emphasizes the pitfalls that may be encountered in attempting to deduce molecular structure from spectra obtained on compounds in a given physical state on the basis of reference spectra obtained on them in a different state.

The authors have evidence that the spectra of some other solid oxygenated derivatives of the long-chain fatty acids, esters, and alcohols may depend on physical state. Additional oxygenated types are being investigated.

Spectra of Liquid Oxirane Compounds. The marked differences between spectra of the solid oxirane compounds in solution and in the solid state suggested a similar comparison between spectra of liquid compounds in the liquid and the solution state. Such a comparison was made for 1,2-epoxydecane, propylene oxide, and *cis*-methyl-9,10-epoxystearate (from methyl oleate). Except for a few slight shifts in the position of absorption maxima, and some changes in width of bands, the spectra of solutions closely resembled those shown in Figures 1, 2, and 3 for these compounds in the liquid state.

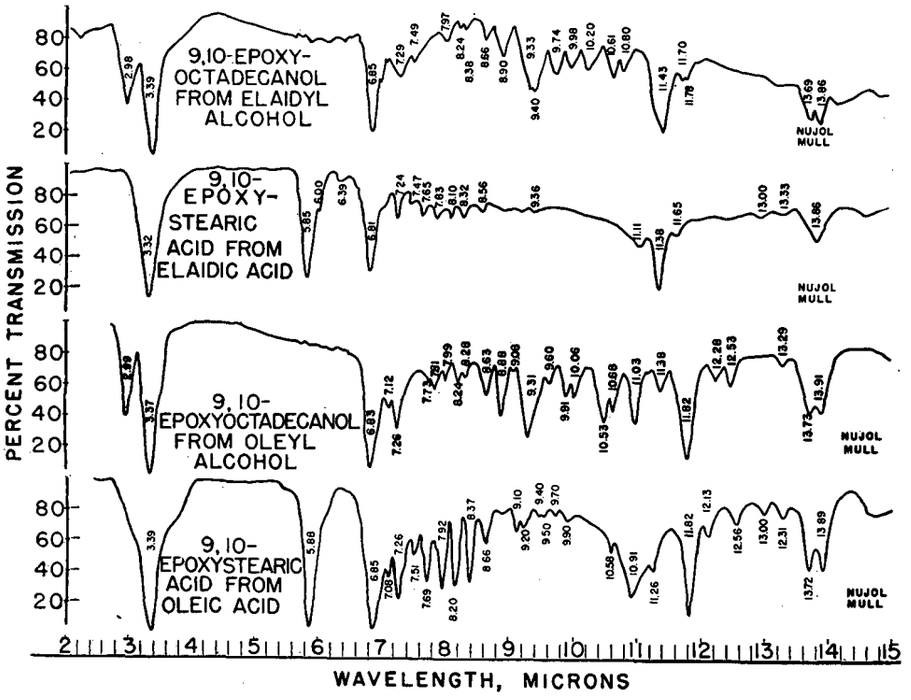

Figure 4. Infrared Spectra of Pure Oxirane Derivatives of Long-Chain 9-Monounsaturated Acids and Alcohols in the Solid State

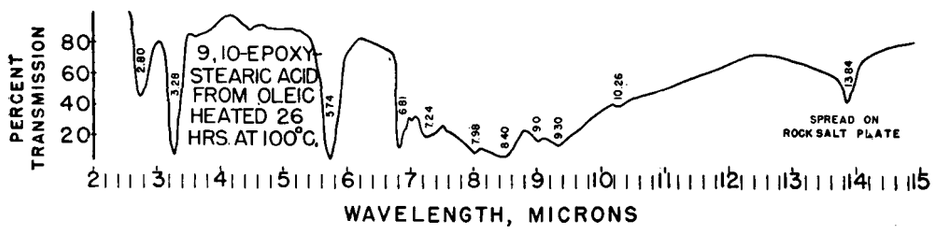

Figure 5. Infrared Spectrum of Thermally Polymerized *cis*-9,10-Epoxystearic Acid

shifted to about 3 microns and intensified. In general, however, the over-all effect was less pronounced than that described above for the solid epoxy compounds.

Effect of Heat on Spectrum of 9,10-Epoxystearic Acid. Thermal polymerization of the 9,10-epoxystearic acids causes the disappearance of oxirane and carboxyl groups and the formation of secondary hydroxyl groups (*8*). The effect of such treatment on the spectrum is seen by comparing the spectrum of *cis*-9,10-epoxystearic acid (Figure 4) with that of a sample which had been heated for 26 hours at 100° (Figure 5). The strong, sharp oxirane ring band near 11.8 microns and the band presumably due to carboxylic O—H bending near 10.9 microns have disappeared. A new band at 2.8 microns shows the formation of alcoholic hydroxyl groups, and the greatly increased absorption in the 8-micron region results from an increase in ester linkages. The sharp band structure characteristic of the crystal state has disappeared, and the CH_2 wagging absorption near 13.8 microns appears as a single band rather than as the doublet observed in the spectrum of the solid.

Other Epoxy Compounds. Figure 6 shows spectra of some

Comparison of Spectra of Stearic Acid and *n*-Octadecanol in the Solid State and in Solution. Again because of the marked effect of physical state on the spectra of the solid epoxy acids and alcohols, the spectra of stearic acid and *n*-octadecanol, previously reported as spectra of solutions (*7*), were redetermined in the solid state. In both, the 13.9-micron band became a doublet, and a few very weak new bands appeared in the 8-micron region in the spectrum of stearic acid and in the 10-micron region in the spectrum of *n*-octadecanol. The 9.5-micron O—H bending band in the spectrum of *n*-octadecanol became sharper, and the 2.7-micron O—H stretching band was

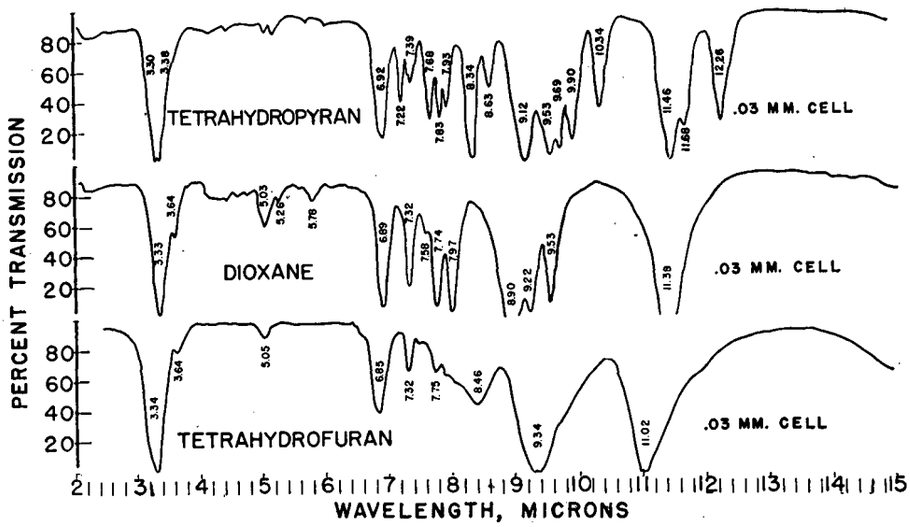

Figure 6. Infrared Spectra of Epoxy Compounds Containing 5- and 6-Membered Rings

epoxy compounds that contain 5- and 6-membered rings. In addition to strong ether absorption in the 9-micron region, these compounds show strong bands in the 11- to 12-micron region. Although additional reference spectra will be required before generalizing, the common band near 11.4 microns in the spectra of tetrahydropyran and dioxane is probably characteristic of the 6-membered heterocyclic oxygen ring in these compounds. Similarly, the 11-micron band in the tetrahydrofuran spectrum is probably characteristic of the 5-membered heterocyclic oxygen ring. The spectra of other compounds that contain similar rings confirm this assumption, but the purity of these additional compounds was not sufficiently certain to justify presentation of their spectra here.

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Infrared Absorption Spectra of Some Hydroperoxides, Peroxides, and Related Compounds

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

The formation of peroxidic substances during autoxidation of unsaturated materials derived from fats and other substances has long been known, but their structure, quantity, and mechanisms of formation have not been adequately established. Infrared absorption spectra of a series of pure hydroperoxides, peroxides, and related compounds from 2 to 15 microns have been obtained and interpreted. On the basis of empirical analysis of the spectra of the hydroperoxides and their parent compounds it has been tentatively concluded that the hydroperoxide group gives rise to a characteristic absorption band near 12 microns. Study of the spectra of peroxides indicates that the peroxide linkage probably gives rise to a strong absorption band in the 10- to 12-micron region, but the frequency corresponding to this band is sensitive to changes in the structure of the groups attached to the peroxide linkage. The spectra are primarily intended to serve as reference data in the application of infrared spectrophotometric methods to the analyses of autoxidation mixtures.

THE formation of peroxidic substances during the reaction of unsaturated materials with oxygen has been known for a long time, but the structure of these reaction products, as well as the mechanism of their formation, has not been completely established. Originally, it was proposed that all the oxygen combined directly with the double bond, yielding a product which was saturated and contained some kind of cyclic peroxide structure (*9–13*). The recent isolation from oxidized nonconjugated olefins of pure α-methylene hydroperoxides, in which the double bond was still intact (*7, 17, 18, 21*), suggested to some investigators that hydroperoxides must be the initial products of oxidation. Consideration of the high energy requirements for hydroperoxide formation, however, coupled with the fact that conjugated compounds containing α-methylene groups autoxidize by addition of oxygen at the double bond, prompted Farmer (*14–16*) to suggest that autoxidation of olefins is universally initiated by addition of oxygen at the double bond of a few molecules only, forming free radicals. Subsequent reaction occurs by chain reactions in which the free radicals attack the α-methylene position. In contrast to this, Hilditch and co-workers (*2, 20*) have reported that at 20° peroxidation of methyl oleate occurs to a large extent at α-methylene groups, whereas at significantly higher temperatures double bond attack predominates. Bolland and Hughes (*5*), however, have reported that in the autoxidation of the polyolefin, squalene, two of the oxygen atoms form a hydroperoxide group and the remaining two form an intramolecular peroxide ring.

These differences in opinion have prompted the authors to consider the use of a physical method in studying the nature of the peroxidic substances formed during the initial stage of oxidation. The main advantage of a physical method in oxidation studies would be the possibility of eliminating the need for isolating labile substances present only in small amounts.

In a relatively short time, infrared spectroscopy has achieved marked success in the qualitative and quantitative determination of oxygen-containing functional groups, in a large number of both short-chain and long-chain compounds (*1, 3, 4, 24, 25*). The literature on organic peroxides, however, is extremely sparse, and in only a few isolated cases has infrared spectroscopy been employed in oxidation studies (*6, 8, 19, 22*). In these investigations, however, no report was made of the use of infrared techniques in determining the nature of the peroxidic substances formed.

Before it is possible to use infrared spectroscopy as a tool in determining the constitution and quantity of the various types of peroxidic substances formed during the autoxidation of compounds derived from fats and other materials, it is necessary to have available reference spectra on pure model compounds. In this paper are reported the infrared absorption spectra of a series of pure hydroperoxides, peroxides, and related compounds from 2 to 15 microns. Interpretation of the spectra is given in the discussion.

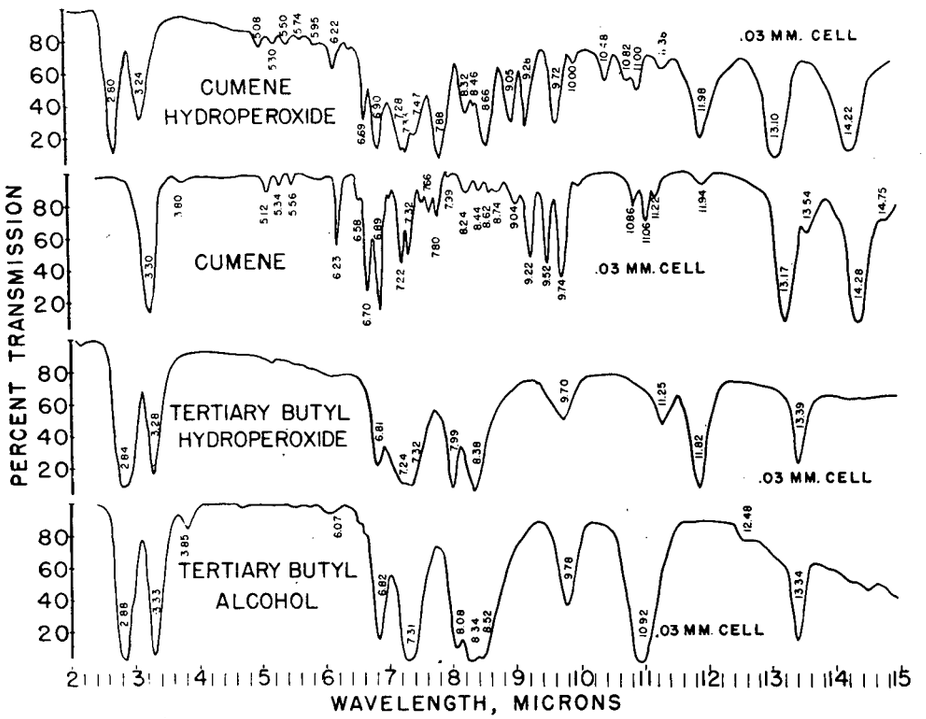

Figure 1. Infrared Spectra of Pure Hydroperoxides and Parent Compounds

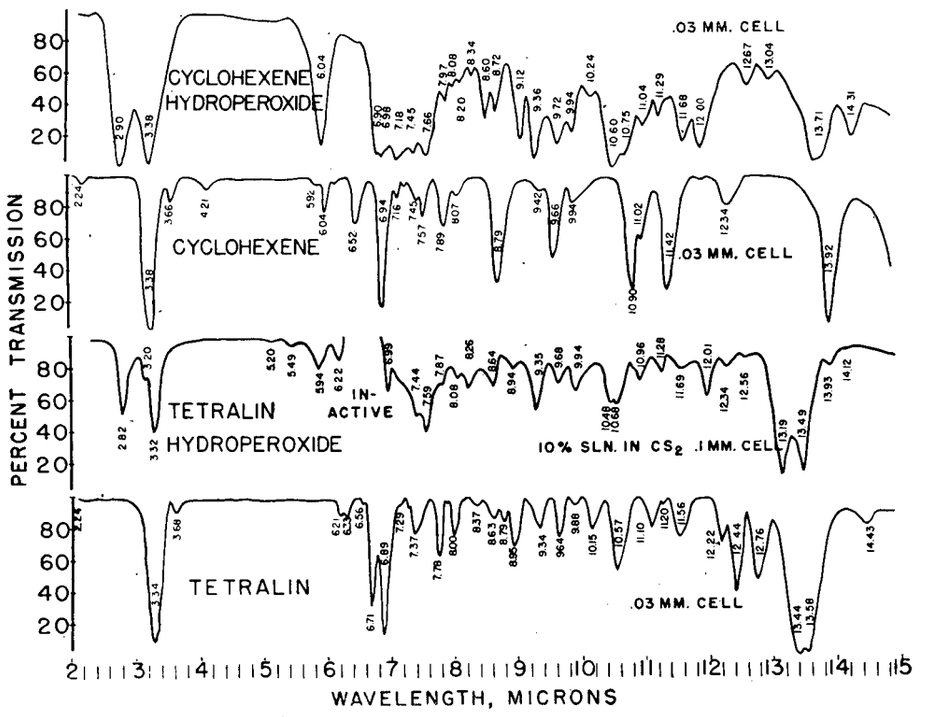

Figure 2. Infrared Spectra of Pure Hydroperoxides and Parent Compounds

EXPERIMENTAL

Spectrophotometer. The spectrophotometer and the technique for obtaining the spectra have been described (*24*). All compounds which were liquid at room temperature were run directly in a standard Beckman liquid cell. Tetralin hydroperoxide, a solid, was run as a 10% solution in carbon disulfide. Benzoyl peroxide, whose solubility in carbon disulfide is comparatively low, was run as an approximately 4% solution.

Materials Used. Tetralin; Tetralin hydroperoxide, melting point 54.0–54.5°; cumene hydroperoxide, boiling point 65° at 0.1 mm. and n^{25}_D 1.5221; cyclohexene; cyclohexene hydroperoxide, boiling point 40–41° at 0.2 mm. and n^{20}_D 1.4892; *tert*-butyl hydroperoxide, boiling point 34.5–35° at 13 mm. and n^{25}_D 1.3987; and di-*tert*-butyl peroxide, boiling point 38.3° at 51 mm. and n^{25}_D 1.3865, were prepared as described in a previous publication (*23*). Cumene (isopropylbenzene), boiling point 152° and n^{20}_D 1.4910, and *tert*-butyl alcohol, boiling point 82° at 748 mm. and n^{20}_D 1.3878, were obtained by efficient fractional distillation of the purest commercial grades. Benzoyl peroxide was the purest commercial grade and was used as received. Methyl oleate hydroperoxide (purity 69%) was supplied by C. E. Swift of the Southern Regional Research Laboratory.

DISCUSSION OF SPECTRA

The spectra of the four pure hydroperoxides studied (*tert*-butyl hydroperoxide, cyclohexene hydroperoxide, Tetralin hydroperoxide, and cumene hydroperoxide) and those of the parent compounds from which they are derived are shown in Figures 1 and 2. Figure 3 shows the spectra of two pure peroxides. Sample form is indicated in the lower right-hand corner and exact wave-length positions of absorption maxima are indicated on each curve.

Common Absorption Bands Attributable to Vibrations of Hydroperoxide Group. Because of the importance of hydroperoxides in studies of oxidation mechanisms, it is of primary interest to examine the spectra of Figures 1 and 2 for absorption bands which might be related to vibrations of the O—O—H (hydroperoxide) linkage. All bands in an infrared spectrum arise from vibrations of the molecule as a

whole, and it is not strictly correct to think in terms of vibrations of a particular linkage within the molecule. Nevertheless, it is a well-known empirically established fact that many functional groups and specific structural units do give rise to bands whose frequencies are substantially independent of the structure of the remainder of the molecule. One band whose wave-length position can almost always be relied on to remain nearly constant with change in molecular structure is that due to O—H stretching vibration. As expected, all four hydroperoxides exhibit this band near 2.8 microns. Comparison with the *tert*-butyl alcohol spectrum indicates no appreciable difference between the hydroperoxidic and the alcoholic O—H stretching frequency. Dilution with carbon disulfide shifts the O—H absorption maximum to shorter wave length in both cases, indicating considerable hydrogen bonding in the condensed state.

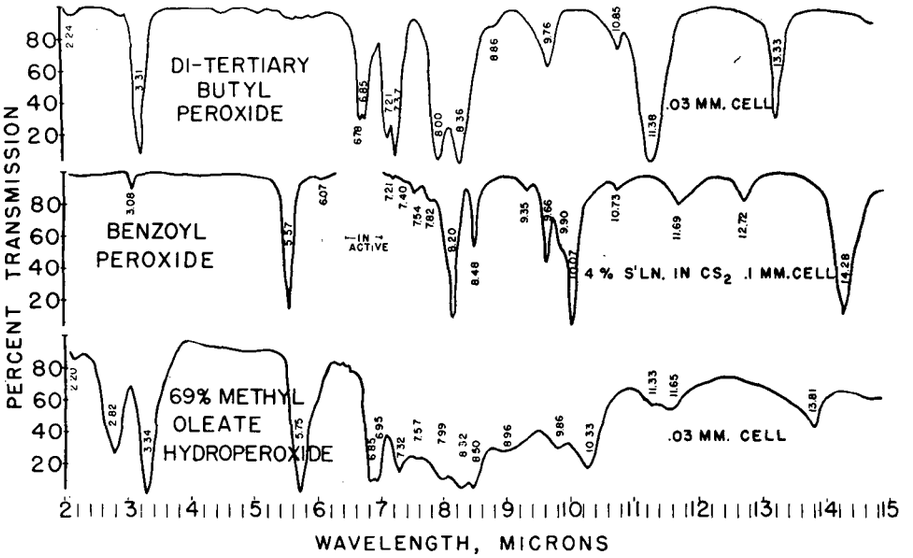

Figure 3. Infrared Spectra of Two Pure Peroxides

In attempting to select an absorption band that might be attributed to vibrations within the hydroperoxide group, or vibrations of that group moving as a unit, it seems best to consider first the spectrum of *tert*-butyl hydroperoxide in relation to that of its parent compound, *tert*-butyl alcohol. These compounds differ only in the substitution of O—O—H for O—H in the molecule, and their spectra are less complex than those of the other compounds of Figures 1 and 2. The following bands which appear in both spectra can be assigned with reasonable certainty as indicated: a band near 2.8 microns due to O—H stretching; bands near 3.3 and 6.8 microns due to C—H stretching and bending respectively; a band near 7.3 microns related to symmetrical deformation vibrations of the methyl groups (*3*, *26*); and two bands at 8 and 8.4 microns related to vibrations of the *tert*-butyl structure (*4*, *26*). The two bands at 9.8 and 13.4 microns are of uncertain origin, but both are present in both spectra. Thus, the only marked consequence of replacing O—H by O—O—H in this case is the disappearance of the strong band at 11 microns (possibly related to OH bending motions in the $-\overset{\diagdown}{\underset{\diagup}{C}}-OH$ structure) and the appearance of two new bands, a weak one near 11.3 and a strong one near 11.8 microns. From this it is concluded that if a band exists between 2 and 15 microns (other than the O—H stretching band) which can be attributed to a vibration within the hydroperoxide group (or of that group moving as a unit), the 11.3- and 11.8-micron bands represent the only possibilities. Because bands arising from vibrations of oxygenated groups are usually strong, the strong 11.8-micron band is the most likely choice.

Having thus established a limited spectral region as a definite possibility, it is now of interest to examine the spectra of the remaining three hydroperoxides in relation to those of their parent compounds in the vicinity of 11.8 microns. On so doing it is found that each of the three does indeed exhibit an absorption band near 12 microns which is absent in the corresponding parent compound.

The argument for assigning the band near 12 microns to a vibration of the O—O—H group is strengthened on further consideration of the cumene and cumene hydroperoxide spectra. In general, the introduction of a substituent into a ring compound brings about marked changes in the spectrum. Because the preparation of cyclohexene and Tetralin hydroperoxides involves the introduction of O—O—H group into a ring, the spectra of these compounds do not retain many of the spectral features characteristic of the parent compounds. In the case of cumene hydroperoxide, however, no new substituent has entered the ring and most of the spectral features of the parent cumene persist in the hydroperoxide spectrum, although some wave-length shifts and changes in intensity are apparent. The following bands which appear in the cumene spectrum are usually observed at approximately comparable wave lengths in a variety of monosubstituted aromatic compounds: the strong bands at 13.2 and 14.3 microns associated with the benzenoid structure; sharp bands near 9.3 and 9.8 microns; two bands near 6.2 and 6.7 microns; the 3.3- and 6.9-micron bands; and the doublet near 7.2 and 7.4 microns. The last bands mentioned are related to C—H stretching, C—H bending, and methyl group deformation, respectively. The additional sharp band seen in the 9- to 10-micron region (near 9.6) may possibly be related to vibrations of the isopropyl group in this compound, although a shorter wave-length range has been assigned to vibrations of this group (*4*, *26*). All these bands persist in the cumene hydroperoxide spectrum, if it can be assumed that the 9- and 9.3-micron bands in the hydroperoxide represent the 9.2- and 9.6-micron bands shifted to somewhat shorter wave length and that the 6.2-micron band has merely undergone a decrease in intensity. Thus it would seem that all but three of the strong bands in the cumene hydroperoxide spectrum can probably be assigned to various vibrations of the cumene residue in the molecule. This leaves three strong bands to account for. Of these three, the two at 7.9 and 8.7 microns (unlike the 12-micron band) are not common to all four hydroperoxides. This leaves the strong 12-micron band as the most probable assignment to a vibration involving the peroxide linkage in the O—O—H group or possibly a bending motion of the hydroperoxide group as a unit.

Because of the complications incident to ring substitution, the assignment of the 12-micron band to a vibration of the hydroperoxide group in cyclohexene hydroperoxide and Tetralin hydroperoxide is less certain. However, in view of the above arguments for the probable reliability of the assignment in the cases of *tert*-butyl and cumene hydroperoxides, together with the fact that the band does appear in all four cases, the authors have tentatively concluded that the presence of the hydroperoxide

group in an organic molecule gives rise to an absorption band in the vicinity of 12 microns.

Aside from the assignments already mentioned, no further interpretation of the complex cyclohexene and Tetralin hydroperoxide spectra will be attempted. One interesting feature of the cyclohexene hydroperoxide spectrum, however, should be noted. While the 6-micron C = C stretching band in the cyclohexene spectrum is only moderately strong, the intensity of this absorption in cyclohexene hydroperoxide has been greatly enhanced. The change in molecular symmetry resulting from substitution of the O—O—H group on a carbon adjacent to the double bond has evidently brought about a marked increase in the infrared activity of this vibration.

Included in Figure 3 is an absorption curve run on methyl oleate hydroperoxide (purity 69%). The broad absorption near 11.7 microns represents a considerable increase over that observed in the spectrum of a sample of pure methyl oleate. Some of this increased absorption probably arises from the hydroperoxide groups present, but conclusions on this point must await the availability of a pure sample of methyl oleate hydroperoxide, which the authors are now attempting to prepare. The failure to observe a stronger increase in absorption in the 12-micron region than was actually obtained might seem surprising. It has been observed, however, in studies (*24, 25*) of other oxygenated long-chain compounds containing oxirane and hydroxy groups, that in the liquid or solution state the absorption bands attributable to these oxygen-containing groups are broad and weak, whereas in the solid state they are sharp and intense. This suggests that infrared spectra of mixtures of oxidation products be determined at low temperatures in the solid state to bring out bands which are broad and weak in the liquid state. The strong O—H stretching band near 2.8 microns arises from hydroperoxidic hydroxyl and other types of hydroxyl groups present in this complex mixture. The strong band at 10.36 microns (not present in methyl oleate) is characteristic of *trans*-octadecenoic acids and esters, thus indicating that geometric isomers may be formed under the oxidizing conditions employed in preparing this mixture from methyl oleate.

PEROXIDES

Only two pure peroxides were available for study at the time of this report. Their spectra are shown in Figure 3.

Di-*tert*-Butyl Peroxide. The spectrum of this compound bears a strong over-all resemblance to those of *tert*-butyl alcohol and *tert*-butyl hydroperoxide. From the discussion of the latter two spectra it will be clear that all bands in the di-*tert*-butyl peroxide spectrum can probably be attributed to vibrations of the alkyl residues, with the exception of the strong band near 11.4 microns and the very weak band at 11 microns. Thus by reasoning similar to that employed in the analysis of the *tert*-butyl hydroperoxide spectrum it may be concluded that the 11.4-micron band is the only band that could possibly be related to vibrations of the peroxide linkage in this molecule.

Benzoyl Peroxide. Although, in view of the above, this compound might have been expected to show an absorption band near 11.4 microns, no such band is present. In the benzoyl peroxide spectrum only three strong bands occur in the longer wave-length region. That at 14.3 microns is related to vibrations of the phenyl ring (*4, 26*) and that at 8 microns is very likely related to a C—O stretching vibration (*4, 24–26*). If, therefore, any band is assignable at the present time to a vibration of the peroxide linkage, the 10-micron band represents the most probable choice. Thus if the highly tentative assignment of the 11.4-micron band in di-*tert*-butyl peroxide and the 10-micron band in benzoyl peroxide to a vibration of the O—O linkage is correct in both cases, the vibration frequency involved must be sensitive to changes in the structure of the two groups attached to the O—O linkage. Although the authors have not yet studied additional pure peroxides, infrared spectra of several additional peroxides (some claimed to be pure and others of uncertain purity) are included in a commercially available catalog of spectra. Examination of these spectra reveals the interesting fact that those containing aryl groups attached to the peroxide linkage (phthaloyl peroxide, *p*-chlorobenzoyl peroxide, and benzaldehyde peroxide) all show a strong band near 10 microns in common with that observed in benzoyl peroxide. Two acyl-type peroxides in this file show common absorption near 9.4 microns. Several others containing widely variable substituent groups show no common band that could be attributed to a vibration of the peroxide linkage.

From these observations, it seems likely that absorption due to the peroxide linkage will vary widely with the nature of the attached groups. In view of the existence of a common band (10 microns) in the four aryl peroxides mentioned above, however, it may be possible to assign a fairly narrow wave-length range to this type of vibration in each of several classes of peroxides whose individual members are closely related.

COMMENTS AND CONCLUSIONS

The potentialities of the infrared method as applied to any type of chemical problem cannot be assessed until spectra of appropriate pure compounds are available for reference. Thus the spectra presented here should be useful in connection with the possible application of this method to a wide variety of problems involving peroxides and hydroperoxides. When sufficient reference spectra of this type are available, it may be possible to distinguish various types of peroxide compounds involved in oxidation and other studies and to determine one type in the presence of others by this physical method of analysis.

ACKNOWLEDGMENT

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RECEIVED May 19, 1950. Presented before the Division of Organic Chemistry at the 177th Meeting of the AMERICAN CHEMICAL SOCIETY, Philadelphia, Pa. Report of a study in which certain phases were carried on under the Research and Marketing Act of 1946. Paper VII in the series "Reactions of Fatty Materials with Oxygen." Paper VI is (*25*).

Xylene Cyanole FF, Redox Indicator

HAZEL M. TOMLINSON, OTTO T. AEPLI[1], AND HELEN M. EBERT[2]
Temple University, Philadelphia, Pa.

In searching for a redox indicator giving a pronounced color change at the equivalence point in determinations of certain metallic ions with ferrocyanide, one of the dyes added to the conventional indicators was xylene cyanole FF. This dye was found to be a suitable redox indicator for titrations of arsenite, ferrocyanide, and ferrous ions with ceric sulfate. The values thus obtained were in excellent agreement with those determined potentiometrically as well as with better known indicators. Attempts were then made to determine the transition and oxidation potentials over a range of acid concentrations.

XYLENE cyanole FF, a dye of the triphenyl carbinol type, is well known through its use in modified methyl orange indicator (*6*). Weinberg (*12*), who patented a method for its manufacture, stated that reducing agents transformed the dyestuff into the leucoform. Brahmajirao (*2*) has reported that it is oxidized irreversibly by potassium dichromate and is unsuitable for the determination of ferrous ion by this oxidant.

EXPERIMENTAL

The authors (*1*) have found that xylene cyanole FF serves as well as does ferroin in the standardization of ceric sulfate with arsenious acid based on the method of Gleu (*5*). In four such analyses using ferroin indicator, the normality of a ceric sulfate solution was 0.05783 ± 0.00001; in four similar titrations in which xylene cyanole FF indicator was used, the average value of the normality of this same ceric sulfate solution was 0.05782 ± 0.00001.

Also, the results obtained in titrations of ferrocyanide ions with ceric sulfate using either eriogreen or erioglaucine, of the same general type as xylene cyanole FF, as well as the more widely recognized redox indicators, diphenylamine and sodium diphenylamine sulfonate, are in excellent agreement with those in which this indicator was used. This is clearly shown in Table I, in which reported values are average values of two determinations; no deviation exceeded 0.6 part per thousand.

Table I. Comparison of Xylene Cyanole FF with Other Indicators in Potassium Ferrocyanide Titrations with Ceric Sulfate

Indicator	Potassium Ferrocyanide, *N*
Xylene cyanole FF	0.04943
Eriogreen	0.04940
Erioglaucine	0.04942
Diphenylamine	0.04949
Sodium diphenylamine sulfonate	0.04943

The comparison of xylene cyanole FF with three other redox indicators used in the titrations of Mohr's salt with ceric sulfate according to the method of Furman and Wallace (*4*) was made. Table II shows that the indicator gives values in agreement with the other indicators the authors employed; duplicate determinations agreed within 0.2 part per thousand.

Table III likewise shows that the deviation in the results obtained by the potentiometric method and those involving the visual end point using xylene cyanole FF are within the limits of experimental error.

Titrations of approximately 0.05 *N* potassium ferrocyanide containing 2 ml. of concentrated sulfuric acid and 1 to 50 drops of indicator, and with final volumes of 95 to 100 ml., required blanks of less than 0.01 ml. of ceric sulfate. In titrations involving the same reagents, concentrations up to 4 *M* in sulfuric acid and up to 1.5 *M* in hydrochloric acid were found to yield almost identical values. In these titrations also, the blanks did not exceed 0.01 ml. of 0.05 *N* ceric sulfate.

Table II. Comparison of Xylene Cyanole FF with Other Indicators in Mohr's Salt Titrations with Ceric Sulfate

Indicator	Mohr's Salt Solution, *N*
Xylene cyanole FF[a]	0.04831
Eriogreen[a]	0.04831
Erioglaucine[a]	0.04831
Ferroin[b]	0.04829

[a] 5 drops of indicator—0.1%.
[b] 3 drops of indicator—0.025 *N*.

Table III. Comparison of Analyses of Ferrocyanide and Mohr's Salt Solutions with Ceric Sulfate, Using Potentiometric Method and Xylene Cyanole FF Indicator

Reagent, *N*	Method	
	Xylene cyanole FF	Potentiometric
Potassium ferrocyanide, soln. 1	0.04964	0.04954
Potassium ferrocyanide, soln. 2	0.05070	0.05069
Mohr's salt soln.	0.04788	0.04788

Knopf (*8*) has reported that the transition potential of xylene cyanole FF is +0.71 volt. It was the authors' plan to redetermine the transition potential and to determine the formal oxidation potential according to the method of Walden, Hammett, and Chapman (*11*) in the case of 1,10-phenanthroline–ferrous complex (*10*). The values which they obtained, as revised by Hume and Kolthoff (*7*), were duplicated by the authors (*3*). In the titrations in which xylene cyanole FF was used, potentials which drifted with time and with accompanying color changes were observed. Changes in indicator and in acid concentration were ineffective in eliminating these drifts. To produce a more pronounced differential curve without introducing a highly colored system, the ferrous-ferric sulfate system in 1 *M* sulfuric acid was replaced by the ferrocyanide-ferricyanide system in 0.01 *M* sulfuric acid. This system exhibits the lowest oxidation potential of those reductants readily available and convenient. Also a saturated calomel electrode replaced the quinhydrone electrode of Walden and his co-workers when the concentration of sulfuric acid was 0.01 *M*, since the latter was found somewhat insensitive to changes in the potential of the accompanying half-cell.

All chemicals used except the xylene cyanole FF were of reagent grade. This dye had been found (*1*) unchanged after adsorption by activated alumina from an ethyl alcohol solution and consequent extraction with water. Five milliliters of 0.004 *M* aqueous solution of dye were present in 100 ml. of the solution used for the titration in each case. The 0.01 *M* ceric sulfate solution used as oxidant was prepared by dilution of a portion of 0.05 *M* stock solution; sufficient sulfuric acid was added in the process of dilution to give the desired acidity in each case. The 0.002 *M* potassium ferrocyanide solution was passed through a Walden reductor immediately before use.

[1] Present address, Pennsylvania Salt Mfg. Co., Philadelphia, Pa.
[2] Present address, Franklin Institute Laboratories for Research and Development, Philadelphia, Pa.

The electrometric apparatus included a student potentiometer, standard Weston cell, and galvanometer of moderately high sensitivity. A reference electrode was prepared for each titration corresponding to the acid concentration of the solution containing the indicator. The half-cells were immersed in a bath held at 25° ± 0.1° C. Calibrated burets and a universal pH indicator assembly were used.

Inasmuch as continued addition of ceric sulfate, acid by preparation, should progressively increase the hydrogen concentration of the solution being titrated, the pH of this half-cell was measured at the several critical potentials being determined. This was done by completing a separate titration only to the desired potential, removing a portion of the solution in the half-cell, and then measuring its pH instrumentally.

The solution for titration which contained potassium ferrocyanide, xylene cyanole FF, and water had a deep blue color with cherry tints. As ceric sulfate solution was added, the cherry color became predominant just past the equivalence point of the oxidation of potassium ferrocyanide to potassium ferricyanide. Blue tints, always present in the cherry, turned to olive, then to gold near the oxidation potential of xylene cyanole FF. However, the red color masked this visual end point. The mixture of red and gold then blended into a bright orange which persisted until, near the end of the titration, the solution became clouded with a fine-grained, white precipitate. This compound has been identified (*4*) as potassium cerous ferrocyanide. Immediately after the predominance of the red color in the solution, constant voltages could not be obtained. With the appearance of the orange color, however, the voltages again became constant. All color changes were gradual; there was no sharp visual end point.

In order to establish the range for possible study of the effect of changes in acid concentration upon the oxidation potential of the indicator, titrations were first carried out in solutions 1 *M* and 2 *M* with respect to sulfuric acid. In the former, suggestions of a differential curve were indicated but, in the latter, curves with only one point of inflection were obtained. Accordingly, determinations were attempted in 0.25, 0.50, 0.75, and 1.00 *M* acid. The transition potentials obtained at these acidities were 1.02, 0.96, 0.97, and 0.98 volt, respectively, while the indicated irreversible oxidation potentials of the indicator were 1.08, 1.11, 1.10, and 1.11 volts.

Potentials were measured using both platinum and gold electrodes according to the standard of reproducibility of Michaelis (*9*). Values agreed within 10 to 20 mv.

CONCLUSIONS

Xylene cyanole FF has been found to compare favorably with the more widely used organic redox indicators in the titrations of Mohr's salt and arsenious acid with 0.05 *M* ceric sulfate as well as in titrations of ferrocyanide in solutions up to 4 *M* in sulfuric acid or 1.5 *M* in hydrochloric acid with the same oxidant. An attempt was made to determine both transition and formal oxidation potentials of the indicator in sulfuric acid solutions.

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Distribution and Type of Sulfur Compounds in Straight-Run Naphthas

J. H. HALE, C. J. THOMPSON, M. G. BARKER, H. M. SMITH, AND J. S. BALL

Petroleum Experiment Station, Bureau of Mines, Bartlesville, Okla., and Petroleum & Oil-Shale Experiment Station, Laramie, Wyo.

THIS report, which is the first concerning the work of American Petroleum Institute Research Project 48A on the separation and identification of sulfur compounds in crude oil, is in the nature of a survey to determine the types of sulfur compounds that may be expected to be found in naphthas from high-sulfur crude oils.

The distribution of free sulfur, hydrogen sulfide, mercaptans (thiols), disulfides, two types of sulfides having different activities, and residual (less reactive) sulfur compounds has been determined for naphthas, 482° F. (250° C.) end point, from 17 typical high-sulfur crude oils of the United States and Middle East. The naphthas included straight-run distillates produced in the laboratory from a number of crude oils by distillation both at atmospheric pressure and at very low pressures (0.5 to 2.0 mm. of mercury), so that comparative data were obtained showing the effect of heating to the temperatures attained in the ordinary distillation of naphtha. The results indicate a wide range in the proportion of the different types of sulfur compounds. However, many of the oils or naphthas contain predominantly sulfides and mercaptans, while relatively few contain appreciable quantities of disulfides. Two are characterized by large quantities of free sulfur. Although many changes in the sulfur compounds present may occur during distillation, the most obvious and interesting one is the almost universal decrease in the content of that unreactive sulfur group called "residual sulfur," in the naphthas produced at atmospheric pressure as compared to those obtained at reduced pressure. In general, this reduction in residual sulfur shows up as sulfide sulfur in the distillate made at atmospheric pressure.

APPARATUS

The apparatus used in the study is shown in Figure 1. Its operation and construction are conventional. Light distillates and hydrogen sulfide were collected in the liquid air trap, from whence, at the completion of the experiment, the hydrogen sulfide was vaporized by warming to room temperature. The liberated hydrogen sulfide was precipitated as cadmium sulfide and the quantity of sulfur was determined by direct weighing in some cases, but iodometrically in most of the experiments.

Table I. Original Selection of Crude Oils

Field	State or Country	Sulfur, Wt. %	Identification No.
Heidelberg	Mississippi	3.75	(2)
Oregon Basin	Wyoming	3.25	(3)
Hawkins	Texas	2.41	(5)
Kirkuk	Iraq	1.93	(9)
Wasson	Texas	1.85	(10)
Wilmington	California	1.39	(12)
Agha Jari	Iran	1.36	(13)
Rangely	Colorado	0.76	(16)
Deep River	Michigan	0.58	(17)

Table II. Additional Crude Oils Selected for Study

Field	State	Sulfur, Wt. %	Identification No.
Santa Maria Valley	California	4.99	(1)
Yates	Texas	2.79	(4)
Goldsmith	Texas	2.17	(6)
Slaughter	Texas	2.01	(7)
Elk Basin	Wyoming	1.95	(8)
Schuler	Arkansas	1.55	(11)
Velma	Oklahoma	1.36	(14)
Midway-Sunset	California	0.88	(15)

PROCEDURE

Distillations at Atmospheric Pressure. In this series of distillations the first cut was made at a vapor temperature in the column of 212° F. (100° C.), and thereafter at every 122° F. (50° C.) rise in vapor temperature until 482° F. (250° C.) was reached, and at this point the distillation was stopped. A distillation rate of 5 to 6 ml. per minute was maintained.

Distillations at Reduced Pressure. In this series of distillations it was intended to produce fractions comparable in weight per cent recovered to those from the atmospheric distillation. To this end, the pressure was reduced in the distillation system until it was as low as was commensurate with the desired distillation rate—approximately 15 to 20 mm. of mercury. After removal of the light ends at room temperature by this process, the pressure was reduced to about 1 mm. of mercury, and heat was applied until a suitable distillation rate—5 to 6 ml. per minute—was reached. The distillation was then continued, taking weight per cent cuts as closely as possible equivalent to those taken in the previous distillation at atmospheric pressure. That this was accomplished with a reasonable degree of success is indicated in Figure 2, where A.S.T.M. D-86 distillation analyses for cut 2, both atmospheric and vacuum, are plotted and compared for two of the crude oils. In both distillations, nitrogen was bubbled at a slow rate through the crude oil throughout the operation, and in all recovered fractions an attempt was made to maintain them in a nitrogen atmosphere until analyses were completed.

Approximately half of the crude oils studied were distilled by both atmospheric and vacuum distillation. For the remaining crude oils studied, only the vacuum distillation procedure was used, and separate fractions were not taken, but only a complete naphtha cut up to a temperature that would correspond to 482° F. (250° C.) at atmospheric pressure was obtained.

CRUDE OILS STUDIED

The crude oils studied in this work are given in Tables I and II; Table I shows those crude oils that were distilled by both the atmospheric and vacuum procedures, while Table II shows those that were distilled to give a complete naphtha cut under reduced pressure.

The distillates were analyzed according to the method developed by Ball (*1*), and the following sulfur groups were determined according to the general method of procedure indicated.

Group	Method of Determination
Hydrogen sulfide	Titration with silver nitrate
Free sulfur	Removal with mercury and determination of difference in sulfur content
Mercaptans	Titration with silver nitrate
Disulfides	Reduction with zinc and acetic acid followed by titration with silver nitrate
Sulfides I	Extraction with mercurous nitrate and determination of difference in sulfur content
Sulfides II	Extraction with mercuric nitrate and determination of difference in sulfur content
Residual sulfur	Sulfur compounds remaining after complete procedure has been carried out

In considering these group sulfur analyses it should be remembered that the method is limited to materials of the gasoline boiling range, as its development showed the reagent to be less reactive to materials of higher molecular weight. The development of methods suitable for the higher boiling ranges, and the consequent analysis of the compounds now reported as residual sulfur, are some of the most important and pressing problems before the project. Sulfides I are commonly considered to be aliphatic and cyclic sulfides, whereas sulfides II are aromatic sulfides and thiophenes. However, some of the aliphatic sulfides, especially those of higher molecular weight, show incomplete removal as sulfides I and are probably removed as sulfides II. There is little evidence of the presence (*5*) of thiophenes in straight-run distillates.

In addition to the group sulfur analyses, a number of other properties were determined, such as density, bromine number, and acid absorption for the distillates from all crude oils investigated.

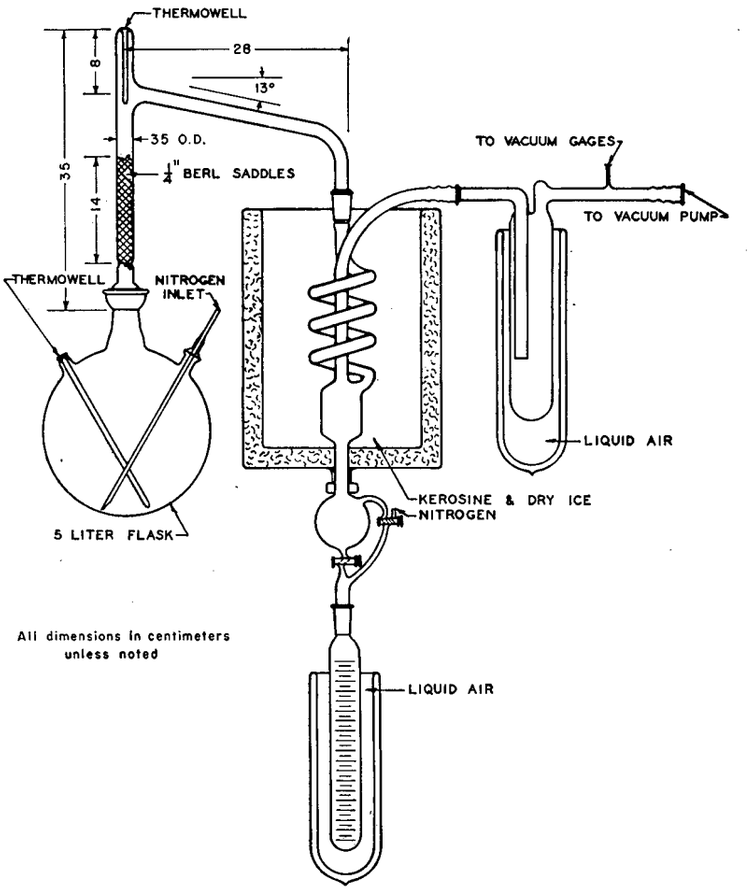

Figure 1. All-Glass Still for Operation at Atmospheric or Reduced Pressure

The purpose of this research was to gain knowledge of the distribution of the types of sulfur compounds in crude petroleum and the effect of temperature on this distribution. It was found that the types of sulfur compounds present in the distillates differ considerably, depending on both the crude oil source and distillation temperature. Sulfur compounds that are not reactive in the scheme of analysis used ("residual sulfur") are predominant and hence little is known of their composition, except that they are changed by heat to more reactive types. The presence of elemental sulfur was established in certain crude oils. The variation in types of sulfur compounds present calls for different methods of refinery processing. The effect of heat in changing types of compounds present is significant, and presence of elemental sulfur is also of importance to refiners. Researchwise the data aided in selection of crude oils and indicated effects of heat and limitations of analytical procedures.

These data are available from the authors to those who may be interested.

DISCUSSION OF DATA ON VACUUM DISTILLATES

Table III presents data for the 482° F. (250° C.) end-point naphtha obtained by distillation of these oils at reduced pressure. For certain of the oils (those where fractions were taken), the data have been obtained by computation from the information for the component fractions. In the case of the other oils, the data were obtained directly on a full-range naphtha cut. It is believed, however, that the data from both sources are comparable. The wide variation in relative amounts of sulfur-compound types among these naphthas is better seen from Figure 3. The lower part of the figure depicts the total sulfur content of each naphtha, and the upper part presents the distribution in terms of the percentage of the various sulfur types within each naphtha. The data shown in Figure 3 are from the analysis of the distillates only, and of these only the distillate from Yates crude oil showed the presence of hydrogen sulfide. However, at least traces of hydrogen sulfide were found in the material caught in the cold traps in virtually all of the vacuum distillations, and Goldsmith and Yates, and to a lesser extent Elk Basin, crude oils yielded copious amounts of hydrogen sulfide. Because of the higher temperatures involved, all atmospheric distillations produced measurable amounts of hydrogen sulfide, both in the cold trap and in the distillate.

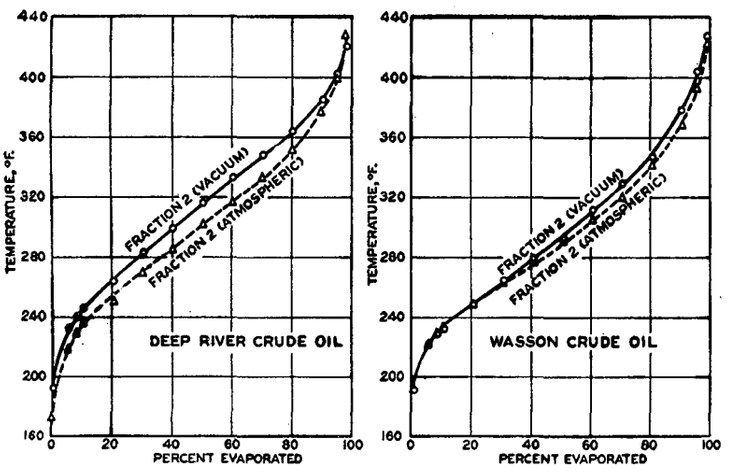

Figure 2. Comparison of Boiling Range of Atmospheric and Vacuum Distillates

Some general interpretations may be drawn from these data, but the investigations are not far enough along to permit many conclusions. In general, at a temperature in the vicinity of 200° to 250° F. (90° to 120° C.) sulfur compounds which are nonreactive to the reagents used in the group analysis appear in the distillate, and their concentration increases rapidly until, in the vicinity of 400° to 500° F. (200° to 250° C.), this residual sulfur constitutes from 20 to 80% of the total sulfur compounds present.

The remaining sulfur compounds (aside from the "residual sulfur") in these distillates produced under vacuum may be used to typify the crude oils from which they were prepared as regards the distribution of sulfur types within the sulfur content of the crude oil. One point of view is to consider the sulfides designated as I and II as one group and the mercaptans and disulfides (because the latter may very probably have originated from the mercaptans) as another group. If the percentage of each of these groups relative to their sum is calculated for each distillate, data such as those in Table IV will result. A study of the table shows that the distillate from Deep River, Mich., is unique in that the sulfur compounds contained are almost entirely of the mercaptan-disulfide group, whereas at the other extreme, naphthas from the Schuler, Velma, Hawkins, Wilmington, Heidelberg, Santa Maria, and Rangely crude oils contain very small quantities of

Table III. Per Cent Sulfur (Based on Total Sulfur) Present as Constituent Indicated in "Vacuum" Distillates

Field No. and Name	Sulfur in Fraction, Wt.	Residual Sulfur	R—S—R II	R—S—R I	R—S—H	R—S—S—R	H_2S	Elemental S
(2) Heidelberg	0.523	80.3	11.7	7.8	0.0	0.2	0.0	0.0
(5) Hawkins	0.377	73.8	14.6	11.1	0.3	0.3	0.0	0.0
(16) Rangely	0.271	72.0	20.3	7.7	0.0	0.0	0.0	0.0
(3) Oregon Basin	1.048	68.2	13.5	15.0	1.7	1.3	0.0	0.3
(12) Wilmington	0.387	66.7	19.9	12.7	0.3	0.5	0.0	0.0
(15) Midway-Sunset	0.385	66.5	26.0	7.3	0.2	0.0	0.0	0.0
(11) Schuler	0.313	66.4	22.7	9.3	0.6	1.0	0.0	0.0
(13) Agha Jari	0.353	65.7	9.6	12.8	8.5	3.4	0.0	0.0
(1) Santa Maria	2.014	58.2	35.5	6.1	0.2	0.0	0.0	0.0
(8) Elk Basin	0.725	54.9	25.1	1.4	11.3	7.2	0.0	0.1
(10) Wasson	0.857	52.6	13.0	11.6	15.3	7.4	0.0	0.1
(7) Slaughter	1.020	48.8	22.5	7.5	10.8	9.2	0.0	1.2
(14) Velma	0.554	43.9	41.5	12.4	1.1	0.7	0.0	0.4
(9) Kirkuk	0.368	41.0	24.7	20.9	7.9	5.5	0.0	0.0
(17) Deep River	0.231	28.6	3.0	0.0	45.9	22.5	0.0	0.0
(4) Yates	1.297	20.5	20.1	9.2	7.5	6.9	1.2	34.6
(6) Goldsmith	0.729	17.3	11.6	9.6	10.6	8.4	0.0	42.5

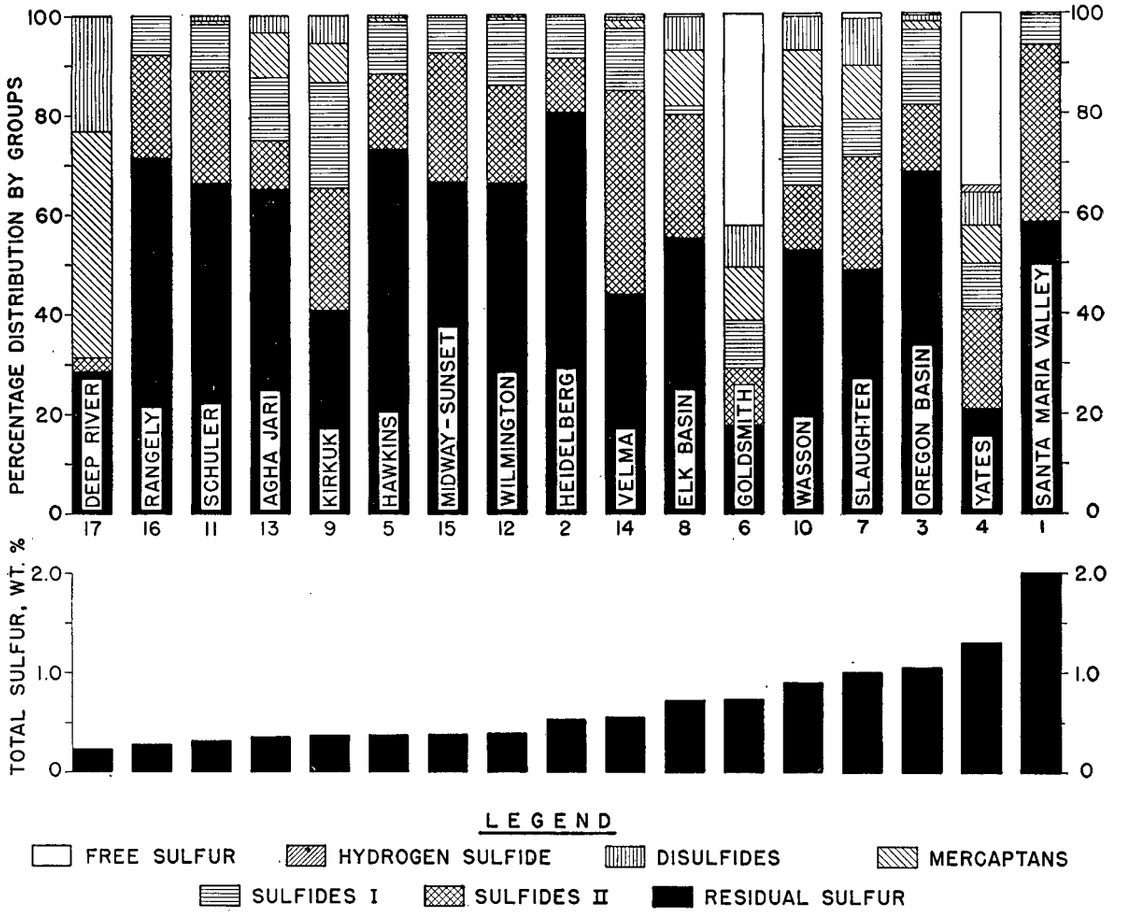

Figure 3. Group Sulfur Analyses of Naphthas from Vacuum Distillations

the mercaptan-disulfide group and large quantities of sulfides. Between these extremes there are several crude oils in which the mercaptan-disulfide group ranges from one third to one half of the total sulfur present in the form of the two groups under consideration. These crude oils include all those from West Texas, as well as those from Elk Basin and Agha Jari. The other crude oils which were studied in this program—namely, Kirkuk and Oregon Basin—fall between the second and third groups of crude oils.

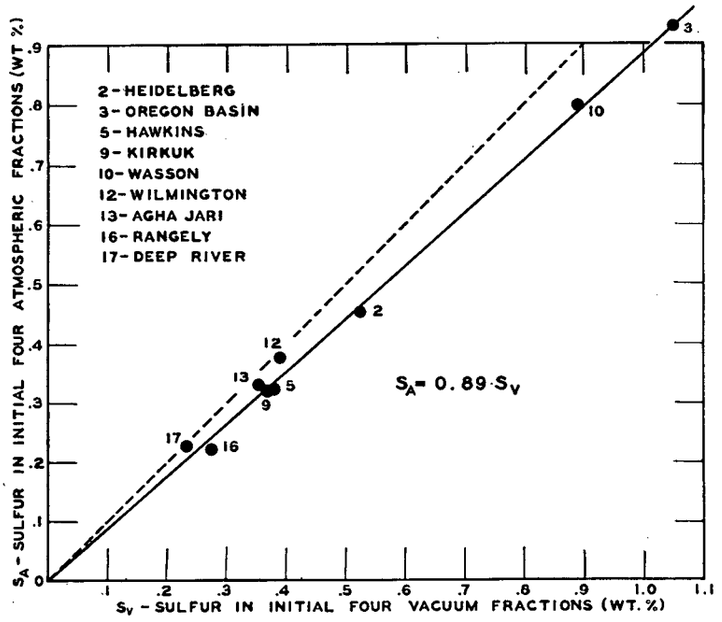

Figure 4. Relationship of Total Sulfur in Atmospheric and Vacuum Fractions

Other peculiarities, of course, are evident—for example, the Yates and Goldsmith crude oils, even under vacuum distillation, yielded distillates that contained large quantities of free sulfur. In fact, in the case of the Goldsmith crude oil the neck of the condenser was stopped with a deposit of free sulfur.

In view of the widespread belief (*3*, *4*) that sulfur in petroleum distillates originates only from the oxidation of hydrogen sulfide by air, the occurrence of elemental sulfur in low-temperature distillates from Goldsmith crude oil was checked in a separate experiment.

Special precautions were taken to ensure the exclusion of all air, either dissolved in the oil or entering by leaks. No gas was used to assist the distillation. The crude oil was admitted slowly through the top of the column into the center of a packed section under a pressure of less than 2 mm. of mercury. This method of charging removed all dissolved gases (air) and light hydrocarbons volatile at room temperature at the pressure used. After the crude oil had been charged in this manner nitrogen was admitted to the vapor space (did not bubble through the oil), so that the cold traps could be emptied. The charge line was then removed and replaced with a thermowell, the apparatus was evacu-

ated to 1 mm. of mercury pressure, and the charge was heated. When the vapor temperature reached 30° C. (corresponding to a pot temperature, estimated from previous experiments, of 60° C.), the capacity of the cold trap was reached and it was necessary to halt the distillation and momentarily to repressure the system with nitrogen as described above while the cold traps were emptied. With the light distillate removed, a vacuum of 0.1 mm. was obtained and the distillation continued without further interruption.

As in two previous distillations of this crude oil, the first appearance of cloudiness in the condensate occurred at a vapor temperature of 100.6° C. Soon a yellow substance appeared in the vapor line and condenser. At the end of the distillation this material was removed, freed of oil, and recrystallized from carbon disulfide. The crystals appeared to be a mixture of monoclinic and rhombic forms and had a melting point of 115.6° C. comparing with 114.5° C. for monoclinic sulfur. The naphtha fractions responded heavily positive to the usual test for elemental sulfur and hydrogen sulfide.

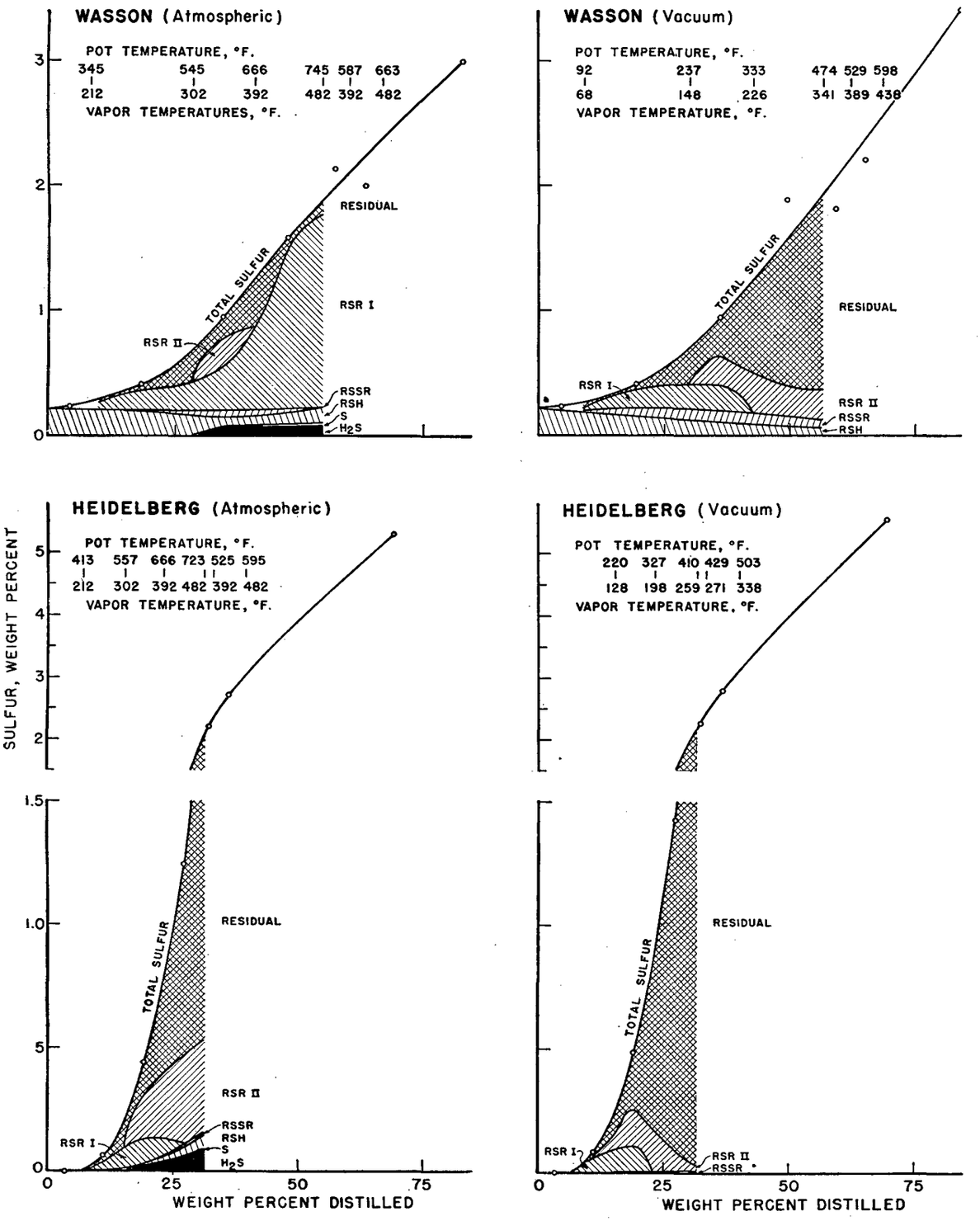

Figure 5. Group Sulfur Analyses from Various Crude Oil Distillates

Table IV. Relationship between Mercaptan Disulfides and Sulfides Groups in Distillates from Various Crude Oils

Crude Oil	Wt. % of Total RSH + RSSR	Sulfides I + II
Deep River	95.8	4.2
Wasson	48.0	52.0
Goldsmith	47.3	52.7
Elk Basin	41.1	58.9
Slaughter	40.0	60.0
Agha Jari	34.7	65.3
Yates	33.0	67.0
Kirkuk	22.7	77.3
Oregon Basin	9.5	90.5
Schuler	4.8	95.2
Velma	3.2	96.8
Wilmington	2.4	97.6
Hawkins	2.3	97.7
Heidelberg	1.0	99.0
Santa Maria	0.5	99.5
Rangely	0.0	100.0

There appears to be no doubt that elemental sulfur appears in vacuum distillates from Goldsmith crude oil at vapor temperatures slightly above 100° C. at 0.1 mm. pressure. Birch and Norris (*2*) observed elemental sulfur at 120° C. during distillation of crude oil. Whether this is caused by elemental sulfur dissolved in the oil, or by decomposition of thermally labile sulfur complexes is not known.

DISCUSSION OF COMPARATIVE DATA FROM ATMOSPHERIC AND VACUUM DISTILLATIONS

The first observation to be made is that there is a slight but apparently consistent difference in the amount of total sulfur contained in distillates produced at reduced pressure and at atmospheric pressure. The total sulfur, as shown in Figure 4, is always slightly higher for the distillate produced at reduced pressure. No reason for this is advanced at present, as all the factors have not been evaluated.

Table V shows the distribution of the sulfur compounds in naphthas produced at atmospheric pressure for nine of the same crude oils for which data were obtained under reduced pressure conditions as given in Table III. Figure 5 presents graphically the results obtained from the group sulfur analyses of the fractions from two of these crude oils, both at reduced pressure and at atmospheric pressure. In this figure, the shaded areas are proportional to the percentage of sulfur in the form designated by the shading assigned to that sulfur group. In this chart an area 1 unit on the vertical axis by 50 units on the horizontal is representative of 0.5% of the crude oil on a weight basis. In the light of the discussion above, it should be remembered that the areas and their form are not exact but the best average that could be assigned with the data available and hence conclusions based on minor variations are not warranted. Probably the most obvious fact to be noted from these figures and the data from the table is the change in "residual sulfur." There is almost always a decrease in the residual sulfur present in atmospheric fractions, that is more or less compensated by a corresponding increase in the quantities of sulfides I and II. In almost all cases, as illustrated by the data for Heidelberg naphtha, sulfide II increases; however, for naphtha from Wasson and Kirkuk crude oils the increase is very definitely in that material which has been designated as sulfide I, as the Wasson data show.

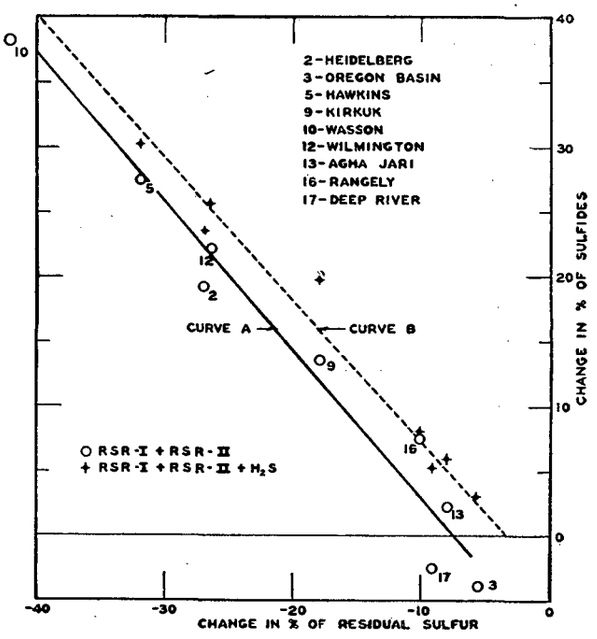

Figure 6. Relationship of Residual and Sulfide Sulfur

This relationship between residual sulfur and total sulfides is shown graphically in Figure 6. In Figure 6, curve *A*, the increase in the percentage of total sulfide is shown as a function of the decrease in the percentage of residual sulfur for each of the distillates under consideration. It can be seen that it approximates a complete balance. If the hydrogen sulfide which was evolved as a gas and not dissolved in the distillate is taken into consideration, an almost perfect correlation results.

CONCLUSIONS

The primary purpose of this paper is to present data showing the distribution of sulfur compounds from distillates produced under ordinary atmospheric conditions and under conditions of minimum decomposition, and to point out one or two of the important changes that are brought about by the relatively small change in temperature between reduced pressure and atmospheric pressure distillation of crude oils.

In any investigation of sulfur compounds or sulfur types in petroleum, one of the most pernicious difficulties encountered is the matter of analysis, and that statement applies to the work here reported. The Ball procedure used in these analyses is reasonably satisfactory on low-boiling fractions, but is inadequate on higher boiling fractions, as indicated by the increase in residual sulfur. Further studies and better analytical methods should resolve some of the questions raised by the present work.

Table V. Per Cent Sulfur (Based on Total Sulfur) Present as Constituent Indicated in "Atmospheric" Distillates

Field No. and Name	Sulfur in Fraction, Wt. %	Residual Sulfur	R—S—R II	R—S—R I	R—S—H	R—S—S—R	H_2S	Elemental S
(2) Heidelberg	0.453	53.4	30.5	8.2	2.4	0.4	4.2	0.9
(5) Hawkins	0.322	41.9	33.2	19.9	2.2	0.0	2.8	0.0
(16) Rangely	0.220	61.8	31.4	4.1	1.8	0.4	0.5	0.0
(3) Oregon Basin	0.932	62.6	11.5	13.0	1.6	0.6	7.0	3.7
(12) Wilmington	0.377	40.3	41.4	13.3	1.3	0.3	3.4	0.0
(13) Agha Jari	0.331	57.7	10.9	13.6	9.1	0.6	3.9	4.2
(10) Wasson	0.799	10.6	6.5	56.2	18.0	3.1	5.1	0.5
(9) Kirkuk	0.320	23.1	23.8	35.3	6.3	2.5	6.3	2.7
(17) Deep River	0.227	19.4	0.0	0.4	44.5	26.9	7.9	0.9

ACKNOWLEDGMENT

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RECEIVED June 6, 1950. Presented before the Division of Petroleum Chemistry at the 117th Meeting of the AMERICAN CHEMICAL SOCIETY, Houston, Tex. Investigation performed as part of the work of American Petroleum Institute Project 48A, "Production, Isolation, and Purification of Sulfur Compounds and Measurement of their Properties," which the Bureau of Mines conducts at Bartlesville, Okla., and Laramie, Wyo.

Determination of Purity and Water Content of Xanthates and Dithiocarbamates

Use of Iodine Reagents

A. L. LINCH

Miscellaneous Intermediates Area Laboratory, Chambers Works, E. I. du Pont de Nemours & Co., Inc., Deepwater, N. J.

Evaluation and development of suitable modifications of methods available for analysis of sodium and potassium xanthates and dithiocarbamates were required to determine accurately the yield, effectiveness of purification procedures, degree of hydration, and final purity of crystalline products isolated. A reliable, rapid method for water determination in crystalline xanthates and dithiocarbamate derivatives by titration with Karl Fischer reagent was developed. Iodine titration of xanthates in an aqueous barium chloride system gave reliable analyses, whereas dithiocarbamate salts required alcohol solutions for best results. The unexpected selective reaction of Fischer reagent with water without oxidation of the active thiol group in either xanthates or dithiocarbamates, and the negligible error introduced by amines and decomposition products, indicate wide application to other oxidation-sensitive materials. Although suitable for xanthate purity analysis, the iodometric titration procedure can be applied with certainty only to relatively pure dithiocarbamate salts, and used only to supplement the more specific gravimetric determination of the thiuram disulfide derived from the iodine oxidation.

A RAPID, reliable, and accurate method of determining water content was required to obtain a materials balance in the analysis of xanthates and dithiocarbamates. The widely employed Dean and Stark procedure (*9, 18*) for the estimation of water by azeotropic distillation with a hydrocarbon entraining agent suffers several serious disadvantages with respect to time required, failure to remove water completely from some hydrates, large sample requirements, and thermal decomposition of many xanthate and dithiocarbamate derivatives. Although the Karl Fischer reagent has been proposed for the rapid determination of xanthate purity directly (*15*, page 401), no reference to its application for the determination of water in these compounds was found. The ease with which iodine is reduced by thiol derivatives, unreacted amines used in the production of dithiocarbamates, by-products and decomposition products such as trithiocarbonates, thiosulfates, and sulfides was expected to interfere with the Fischer titration.

Of the methods proposed for determination of xanthate and dithiocarbamate purity, direct titration with standard iodine solution offered the most promise as a rapid and accurate procedure, if interferences from by-products and decomposition products could be controlled (*2–5, 7, 13, 14*). In the authors' experience, titration with heavy metal salts (iron, nickel, lead, copper, or zinc) was not entirely satisfactory, owing to formation of basic salts and coprecipitation (*1, 8, 16*). Improved accuracy obtained by isolation and determination of the metal content of the insoluble copper salts not only entailed laborious and time-consuming operations, but also failed to separate dithiocarbamates from impurities (*6, 10, 11*). Quantitative decomposition of sodium or potassium ethyl xanthate with acid was not generally applicable to other xanthates or to dithiocarbamates (*12*). Estimation of purity by sulfur analysis (Parr bomb) was likewise time-consuming and subject to errors introduced by by-products, especially sulfides and disulfides. Gravimetric determination of the insoluble disulfides (dixanthogens and thiuram disulfides) from iodine oxidation required more time than direct titration, but good results could be obtained when conditions for quantitative recovery were developed.

KARL FISCHER MOISTURE DETERMINATION

The determination of water by direct titration in chloroform with Karl Fischer reagent required no specialized technique to obtain accurate results rapidly with a visual end point, when the usual precautions to exclude atmospheric moisture were observed.

Reagents. Karl Fischer reagent. Dissolve 404 ml. of C.P. pyridine in 1000 ml. of anhydrous methanol and add 127 grams of iodine crystals. When dissolved, chill in ice and add 100 grams of sulfur dioxide as a gas under the surface of the liquid. Standardize against standard water solution in methanol (*15*, page 65).

Chloroform, C.P. reagent.

Procedure. Measure 25 ml. of chloroform into a dry 125-ml. Erlenmeyer flask (assembly described on page 72 of *15* is recommended for this determination), add Karl Fischer reagent dropwise through a two-hole stopper until a definite visual end point is reached (2 to 3 ml.) with vigorous swirling to wash down sides of the flask, and quickly add 0.5 to 5 grams of sample weighed to

Table I. Accuracy of Moisture Determination

(Direct titration, visual end point)

Compound	Solvent	Water Added[a], %	Water Found, %	Relative Error, %
Sodium di(β-hydroxyethyl)dithiocarbamate	Methanol	None	1.7	....
	$CHCl_3$	None	1.7	....
	Methanol	10.4	10.1	−2.9
		15.3	15.3	Nil
		33.6	33.6	Nil
Diethanolamine		None	0.65	....
	$CHCl_3$	None	0.63	....
	Methanol	12.1	11.8	−2.5
		19.0	18.5	−2.6
Sodium thiosulfate		36.3	41.3	+13.8
	$CHCl_3$	36.3	38.9	+7.2
Back-titration	CH_3OH	36.3	41.4	+14.1
Sodium sulfite (66)–sodium sulfate (34) mixture	$CHCl_3$	41.2	41.6	+1.0

[a] Includes water present in initial sample.

three decimal places. Samples containing more than 10% moisture should be reduced to 0.5 to 1.0 gram in size, while less than 1% moisture requires a 4.0- to 5.0-gram sample for accuracy. Titrate to a permanent end point with constant swirling.

Calculation

$$\% H_2O = \frac{\text{ml. of Fischer reagent} \times \text{factor (as grams of } H_2O \text{ per ml.)} \times 100}{\text{sample weight}}$$

Discussion. The average accuracy of ±1% was not affected by the presence of such reactive amines as diethanolamine and ethylenediamine (Table I). Although the relative accuracy of the method applied to dibutylamine, ethylenediamine, α-pipecoline, and monoethylaniline was not determined, direct titration results and addition of these amines to the corresponding dithiocarbamate derivatives indicated no interferences. However, titration of diethanolamine to which known amounts of water were added has shown that the Fischer reagent can be applied to water analysis of amines which react with iodine, as well as xanthates and dithiocarbamates (Table I).

Not only has the determination of water added in known amounts to dithiocarbamates fallen within the useful limits of accuracy, but a majority of the sums of purity by sulfur analysis and water content by Fischer titration (material accounted for) for 39 xanthate (Table II) and dithiocarbamate (Table III) determinations were within the range 98 to 101%. Because many of these compounds were relatively unstable, were converted appreciably to disulfide derivatives by atmospheric oxidation, and were difficult to separate from by-products, calculation of purity from total sulfur content contributed a majority of the deviations from 100%. Sodium thiosulfate, a xanthate decomposition product, introduced 5 to 10% (of the amount present) positive error, but sulfites did not produce high results. Sulfide contamination of crystalline xanthates and dithiocarbamates was not sufficient to require an evaluation of its effect on accuracy.

IODOMETRIC DETERMINATION OF XANTHATE PURITY

Satisfactory determination of xanthate purity by conventional titration of an aqueous solution with standard aqueous iodine has been obtained in the absence of more than traces of by-products and decomposition products such as sulfites, thiosulfates, sulfides, and thiocarbonates, which were easily detected and results modified accordingly. Better accuracy was attained, at the expense of time consumed, by pretreatment with barium chloride.

Reagents. Aqueous iodine, 0.1 *N* (450 grams of potassium iodide plus 230 grams of iodine diluted to 18 liters).

Barium chloride, 10% (10 grams dissolved in distilled water and diluted to 100 ml.).

Starch indicator solution, 10 grams of corn starch (Argo) heated in 2 liters of water to boiling. Let stand overnight and decant off the clear supernatant solution.

Procedure. Dissolve a 1-gram sample weighed to three decimal places in 50 ml. of distilled water, add 5 ml. of barium chloride solution, stir thoroughly, and let stand 2 hours. Then add 150 ml. of distilled water and titrate with 0.1 *N* aqueous iodine almost to an end point, add 5 ml. of starch indicator solution, and finish the titration to a permanent blue or red end point (color somewhat dependent on the nature of the xanthate).

Calculation

$$\% \text{ purity} = \frac{\text{ml. of 0.1 } N \text{ iodine} \times \text{factor} \times \text{mol. wt.} \times 0.1}{\text{sample weight}}$$

These xanthates were crystallized from the alcohol from which they were synthesized, but, with one or two exceptions, received no special purification. Most of the samples were stored several weeks to more than a year before analysis. Several were titrated in an alcohol system, but in every case an unreasonably high value was obtained. Titration with copper or nickel salts also gave high results.

Discussion. The rapid iodometric method for the estimation of the purity of sodium or potassium xanthate derivatives in aqueous medium in the presence of barium chloride has shown ±1% average accuracy, which is better than can be realized from calculation of purity from total sulfur content (Table II). Suitable conditions were not found for the suppression of interference from sulfides and thiocarbonates by precipitation with lead in the presence of tartrates. Therefore, the longer method of Grete (*6*, *10*) must be used for samples contaminated with these impurities. Combination of total sulfur analysis, and iodine titration directly and in the presence of barium chloride, yielded reasonably reliable data on the sulfite and dixanthogen content of a sample, inasmuch as the difference in iodometric titrations was produced by sulfites and the "excess" purity by total sulfur was derived from the disulfide derivative which did not titrate. Therefore, in Table II positive errors were interpreted

Table II. Analysis of Xanthates

No.	Structure ROCSK (Na) ‖ S	Purity, % Iodine titration	Purity, % Sulfur anal.	Relative error, %	Fischer Titration, % H_2O	Materials Balance, % Iodine Purity + H_2O
1	K methyl	97.4	96.8	+0.6	2.4	99.8
2	K ethyl	92.7	93.5	−0.9	4.8	97.5
		95.2	96.3	−1.1	2.0	97.2
3	Na ethyl	89.7	89.6	+0.1	9.8	99.5
4	K *n*-propyl	100	99.5	+0.5	0.5	100.5
5	Na *n*-propyl	80.0	77.8	+2.8	20.0	100
		72.8	73.6	−1.1	21.4	94.2
		97.4	99.0	−1.6	2.6	100
6	K isopropyl	97.3	96.5	+0.8	2.1	99.4
7	K *n*-butyl	94.1	95.6	−1.6	4.1	98.2
8	K *sec*-butyl	92.6	90.6	+2.2	5.9	98.5
	With $BaCl_2$	89.0	...	−1.8	..	...
9	K isoamyl	95.3	99.3	−4.0[a]	4.2	99.5
10	K 1,3-dimethylbutyl	96.4	98.0	−1.6	4.2	100.8
11	K 1-methyl-*n*-amyl	79.7	81.7	−2.5	17.3	97.0
12	K octyl	76.7	78.2	−1.9	6.2	82.9
13	K cyclopentyl	96.4	96.7	−0.3	2.8	99.2
14	K cyclohexyl	79.0	80.6	−2.0	5.7	84.7
15	K allyl	93.1	100	−6.9	3.7	96.8
16	K methallyl	99.3	99.2	+0.1	4.2	103.5
	With $BaCl_2$	97.7	...	−1.5	..	101.9
17	K β-ethoxyethyl	91.5	87.4	+4.7	6.8	98.3
	With $BaCl_2$	87.3	...	−1.1	..	94.1

[a] Contains water-insoluble oil.

Table III. Analysis of Dithiocarbamates

No.	Structure $R_2N-C(=S)-S\ Na$	Purity, % Nitrogen	Purity, % Iodine titration[a]	Purity, % Sulfur	Relative Error, %	Fischer Titration, % H_2O	Materials Balance, % Iodine Purity + H_2O
1	Dimethyl	76.7	...	78.5	+0.6	21.2	100.2
			79.7[b]	78.5	+1.5	20.8	100.5
2	Diethyl	73.8	74.8	74.8	0.0	22.3	97.1
		75.3	76.4	76.5	−0.1	23.4	99.8
3	Diisopropyl	76.7	80.0	83.0	−3.6	18.2	98.2
4	Di-*n*-butyl	93.4	87.8	92.6	−5.2[c]	6.0	93.8
5	Diamyl	95.2	94.5	94.9	−0.4	2.7	97.2
6	Dioctyl	86.9	77.1	87.1	−11.5	13.8	90.9
7	Bis(2-hydroxyethyl)	97.4	98.3	99.4	−1.1	1.7	100
8	Phenylethyl	75.8	76.5	80.1	−4.5[d]	21.9	98.4
9	Pentamethylene	75.9	76.8	78.5	−2.2	17.3	94.1
10	Pentamethylene[e]	100.2	100.2	100.3	−0.1	1.0	101.2
11	α-Pipecoline	84.0	85.9	85.7	+0.2	13.4	99.3
12	γ-Pipecoline	...	75.0	77.0	−2.6	22.0	94.0
13	2-Pyrimidyl C_2H_5OH	93.1	90.0	90.1	−0.1	7.7	97.7
14	Ethylene-bis	68.7	68.9	70.0	−1.6	29.5	98.4
		69.6	72.0	70.6	+2.0	29.5	101.5
15	Piperazyl	80.3	85.4	83.6	+2.2	12.8	98.2
16	Piperazylene	86.5	86.3	84.4	+2.3	11.9	101.2

[a] Alcohol solution titrated with alcoholic iodine.
[b] Purity by nickel ion titration = 75.3%.
[c] Sample contains 7.0% insoluble material.
[d] Sample contains 2.8% insoluble disulfide; therefore, corrected error = 0.7%.
[e] Piperidinium salt.

as sulfite (corrected by barium chloride) and negative errors as dixanthogen contamination.

IODOMETRIC DETERMINATION OF DITHIOCARBAMATE PURITY

The best conditions for determination of dithiocarbamate purity required titration of an alcohol solution of the sample with standard alcoholic iodine reagent. The appearance of a bright yellow color in the absence of starch indicator provided the most satisfactory end point which, in most cases, could be verified by starch solution as an outside indicator. However, the starch-iodine blue color faded very quickly, and required practice to locate the correct end point. Barium chloride employed in xanthate titrations could not be used to suppress impurities, as the partial precipitation of insoluble barium dithiocarbamates introduced serious errors.

Reagents. Ethyl alcohol, Formula 2B. Alcoholic iodine, 0.1 *N* in 2B alcohol.

Procedure. Dissolve a 1-gram sample weighed to three decimal places in 150 ml. of 89 to 90% ethyl alcohol (insoluble matter indicates presence of the thiuram derivative), and titrate to the first appearance of a bright yellow color which persists at least 1 minute at the end point. The end point can be confirmed by spot test in starch solution on a white spot plate. The color is fugitive, however, and with some dithiocarbamates requires overtitration for a definite test.

Calculation

$$\% \text{ purity} = \frac{\text{ml. of 0.1 } N \text{ iodine} \times \text{factor} \times \text{mol. wt.} \times 0.1}{\text{sample weight}}$$

Good confirmatory results have been obtained by titrating in an aqueous system, filtering off the insoluble thiuram disulfide derivative on a tared Gooch crucible, washing carefully with water, and weighing after drying to constant weight at 80° C.

Calculation

$$\% \text{ purity} = \frac{\text{wt. of thiuram disulfide} \times \text{mol. wt. of dithiocarbamate} \times 200}{\text{sample weight} \times \text{mol. wt. of thiuram disulfide}}$$

Test for Sulfides. Dissolve approximately 0.1 gram of sample in 10 ml. of cold water, and add a few drops of a 2% aqueous solution of sodium nitroprusside. Immediate appearance of an intense red-violet color indicates presence of appreciable quantities of sulfides which will interfere with iodometric analysis.

These dithiocarbamate derivatives were crystallized from water, but, with the exception of 13 to 16, received no further purification treatment. Samples 4 to 6 represent commercial materials.

Discussion. The iodometric analysis of water-soluble dithiocarbamate derivatives has encountered several sources of error for which compensating techniques have not been developed; therefore, its use for purposes other than a quick approximation (±2% under favorable conditions), or to furnish auxiliary data, cannot be recommended. Conditions which permit titration of xanthate samples containing sulfites were not applicable, and the elimination of sulfides by selective lead precipitation was not successful. However, the iodometric method appeared to be as accurate as titration with heavy metal reagents, and applied to crystallized dithiocarbamates, more accurate than purity by sulfur analysis (Table III). Titration results lower than sulfur purity were corrected by gravimetric determination of the water-insoluble thiuram disulfide content. Although reliable results were obtained in some cases by titration of an alcoholic dithiocarbamate solution with aqueous iodine (Table IV), in general the use of alcoholic iodine titrant was found necessary to avoid non-stoichiometric relationships encountered in some aqueous systems.

Reactive amines (diethanolamine, ethylenediamine) introduced errors considerably greater than the equivalent amount present (calculated as amine), and relatively "inert" amines, such as dibutylamine, yield small, but significant, error. A few (monoethylaniline and pipecoline) exert a negligible effect. Probably the most accurate method for determination of purity is the gravimetric procedure based on oxidation to the water-insoluble thiuram disulfide (Table IV).

CONCLUSIONS

Application of the Karl Fischer titration for the determination of water to the analysis of xanthates and dithiocarbamates has furnished an improved method for the rapid and accurate determination of water of hydration as well as extraneous water, with a relative accuracy of ±1%. Most alcohols and amines

Table IV. Effect of Titration Technique on Accuracy

(Dithiocarbamate purity by titration with iodine. Sodium phenylethyl dithiocarbamate)

Solvent for Sample	Solvent for Iodine	Method for Detecting End Point: Inside yellow color, no starch	Method for Detecting End Point: Spot plate starch solution
Ethyl alcohol (2B)	Ethyl alcohol (2B)	75.2	76.5
	Aqueous	75.0	76.3
Water	Aqueous	78.6	79.5

Purity by sulfur determination (corrected for 2.8% water insoluble disulfide derivative) = 77.0
Gravimetric purity (calculated from weights of disulfide produced by iodine oxidation) = 75.1

Table V. Effect of Impurities on Accuracy

(Dithiocarbamate purity by titration with iodine)

Derivative	"Impurity" Added: Compound	"Impurity" Added: %	Purity Found, %	Relative Error, %
Sodium bis(β-hydroxyethyl)	None	...	98.3	....
	Diethanolamine	16.7	129	+31.3[a]
Sodium α-methylpentamethylene	None	...	78.3	....
	α-Pipecoline	12.8	81.0	+3.3
Sodium phenylethyl	None	...	76.5	....
	Monoethylaniline	18.5	76.3	−0.3
Sodium diamyl	None	...	94.5	....
	Dibutylamine	14.5	102.2	+8.2
		16.0	106.9	+13.1
Disodium ethylene-bis	None	...	72.0	....
	Ethylenediamine	20	135.1	+88[b]

[a] 16.3 calculated as diethanolamine.
[b] 18.8 calculated as ethylenediamine.

from which these compounds are synthesized did not react with the reagent, and decomposition products such as thiosulfate and sulfites introduced only minor errors.

Titration of aqueous sodium and potassium alkyl xanthates with aqueous iodine in the presence of barium chloride yielded results more rapidly and of a higher order of accuracy in the absence of sulfides and thiocarbonates than purity calculated from total sulfur content or titration with a heavy metal salt reagent.

The iodometric procedure for the estimation of dithiocarbamate purity can be recommended only as a rapid approximate method, or as a source of auxiliary information, except in cases where interferences are known to be absent. However, reliable data can be obtained from most crystallized products, and valuable information relative to degree of oxidation to the thiuram-disulfide derivatives can be estimated.

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Extraction and Purification of Nordihydroguaiaretic Acid

JOHN O. PAGE, *Agricultural & Mechanical College of Texas, College Station, Tex.*

It was desirable to work out an accurate method for the quantitative determination of nordihydroguaiaretic acid in creosote bush (*Larrea divaricata*). Isopropyl ether or isopropyl ether–carbon tetrachloride mixtures, rendered peroxide-free by a preliminary washing with aqueous sodium bisulfite solution, quantitatively extract the nordihydroguaiaretic acid from the creosote bush leafy material. The solvents are distilled and recovered. Extractions of the tarry residues with boiling distilled water quantitatively separate crude nordihydroguaiaretic acid (melting point, 176–180° C.) from the tarry or resinous residues. Yields of 2.14 to 2.35% of the crude were obtained from large samples of fresh green, machine-threshed, creosote bush. The percentage of nordihydroguaiaretic acid in the crude was not determined, but several methods were tried in order to evaluate the purity of crude nordihydroguaiaretic acid with accuracy. Methods of purification are given. Nordihydroguaiaretic acid is principally useful as a food antioxidant. It is separated from creosote with some impurities and weighed in the crude form. The resulting data are proximate assay recoveries, as the purity of the crude material, that was separated and recovered by this method, was not determined.

THE phytochemical study of creosote bush (*Larrea divaricata*) was made by Waller (*37*, *38*), who obtained nordihydroguaiaretic acid from this plant. Waller extracted the crude chemical with 95% ethyl alcohol and then recrystallized the nordihydroguaiaretic acid from hot dilute aqueous acetic acid or from sodium bisulfite solutions. In an attempt to separate the pure phenol from a large quantity of the plant extract by steam distillation, Waller (*37*) obtained impure crystals which were suspended in the water above the settled plant extract. He then found that water itself did not separate the pure nordihydroguaiaretic acid from the plant extract (or tar), and consequently used aqueous acetic acid or sodium bisulfite solutions to purify the chemical.

The extraction of nordihydroguaiaretic acid is the subject of seven patents (*1*, *9–14*) and one publication (*8*); the antioxidant properties of this chemical have been substantiated and demonstrated in twenty-three publications (*2–5*, *15*, *16*, *18–25*, *27–35*). The first of these patents (*10*) disclosed the process by means of which 2.50 to 2.66% yields of "90 to 100%" pure nordihydroguaiaretic acid were obtained from the creosote bush.

The creosote bush is first extracted with an aqueous solution containing, usually, 5% sodium hydroxide and 2 or 2.5% of sodium hydrosulfite ($Na_2S_2O_4$). This alkaline extract is then acidified with concentrated hydrochloric acid solution, after which step a yellow-brown viscous, curdy, solid crude material separates both at the top of the acidified extraction liquor and at the bottom of the tank. This is industrial practice in separating the crude nordihydroguaiaretic acid from the creosote bush material.

This crude curdy material is purified by various means, including the method first described in a United States patent (*10*)—viz., the crude curdy nordihydroguaiaretic acid sludge may be dissolved in an aqueous-alcoholic solution or medium. The nordihydroguaiaretic (together with some impurities) may be taken out or dissolved out of the aqueous-alcoholic environment with a water-immiscible solvent such as diethyl ether or isopropyl ether. The diethyl ether or isopropyl ether thus serves as a convenient solvent for the nordihydroguaiaretic acid (and associated impurities) and as a preliminary step before purification of the acid.

A patent (*11*) claims that diethyl ether or isopropyl ether may be used to obtain a crude extract, containing nordihydroguaiaretic acid, from creosote bush. However, it discloses no useful means or devices which can be employed to translate or convert the hazardous laboratory procedure of extraction with isopropyl ether into a useful art.

The purposes of the present work are to gain accurate quantitative data on the amount of nordihydroguaiaretic acid present

Table I. Yield of Crude Nordihydroguaiaretic Acid

Sample		First Solvent Tank		Second Solvent Tank		Aqueous Filtrate Liquor		Total Crude NDGA	
	G.	M. p., ° C.	%	M. p., ° C.	%	M. p., ° C.	%	G.	%
Old, dry	4,225	177–181	1.34	None[a]		.....	..	56.9	1.34
Fresh, green machine-threshed	10,982	176–180	1.52	175–179	0.33	170–175	0.29	235.3	2.14
Fresh green, machine-threshed	4,700	177–180	1.56	172–178	0.16	178–182	0.58	108.1	2.30
Fresh green, machine-threshed	4,200	177–181	1.48	162–169	0.13	175–179	0.74	98.8	2.35

[a] Boiling H_2O extractions.

in the creosote bush, starting with significant quantities of the creosote bush as samples, and if possible, to improve the method of extraction by lowering the present cost of extraction and purification of this chemical.

Water solutions containing 0.5% of the sodium sulfonates of cresylic acid quantitatively extracted the chemical from the creosote bush, but, in taking the chemical out of its water solution with cresylic acid, some emulsion phase was formed. This method of extraction was abandoned, although the quantity of emulsion phase was reduced by the use of calcium chloride at low concentrations. Extractions with cyclohexanol-water and isopropyl alcohol–water solutions were productive only of low yields in a colloidal state. Treatment with hot aqueous sodium bisulfite solutions did not dissolve any nordihydroguaiaretic acid from the creosote bush material. However, isopropyl ether or isopropyl ether–carbon tetrachloride mixtures quantitatively extract the nordihydroguaiaretic acid from the cresote bush leafy material.

Earlier work by the writer (*26*) had shown that the nordihydroguaiaretic acid (melting point 184–185° C.) is destroyed quantitatively when solutions in 95% ethyl alcohol, U.S.P. diethyl ether, or isopropyl ether are permitted to stand overnight or for 2 or 3 days. This difficulty disappeared entirely when the solvents were purified and redistilled. The 95% ethyl alcohol was first treated with silver oxide, then redistilled, while the diethyl ether and isopropyl ether were treated with dilute sulfuric acid–potassium permanganate solutions before redistillation. It is probable that aldehydes and peroxides, apparently present in the unpurified 95% ethyl alcohol and in the unpurified ethers, respectively, accounted for the total destruction of the nordihydroguaiaretic acid.

With this experience in mind, the practice was established of washing the isopropyl ether–carbon tetrachloride mixture with a dilute aqueous solution of sodium bisulfite, leaving the mixture wetted with the sodium bisulfite solution. The preliminary washing with dilute aqueous sodium bisulfite solution was always performed, even though the fresh isopropyl ether yields no evidence of the presence of peroxides when tested for free iodine with acidified potassium iodide solution. However, upon standing for some time in contact with air, there is evidence of peroxide formation in the isopropyl ether or the mixtures of isopropyl ether with carbon tetrachloride. For example, after several weeks' standing, 150 ml. of an isopropyl ether–carbon tetrachloride mixture liberated iodine equivalent to 0.45 ml. of 0.0525 *N* sodium thiosulfate solution. Another sample of this same mixture was washed with a small volume of 1% sodium bisulfite solution, and then the mixed solvents were washed several times with distilled water. When so treated this mixture no longer liberated any iodine from acidified solutions of potassium iodide, indicating freedom from peroxides.

A quantity, such as 2400 grams, of the threshed creosote bush is lowered into a tankful of the cool, sodium bisulfite–washed, solvent mixture, in a closed fine-mesh steel basket. After extraction periods up to 4.5 hours, the extracted sample is withdrawn from the solvent solution, drained, and then placed in a tank containing another portion of the cool, sodium bisulfite–washed, isopropyl ether–carbon tetrachloride solvent. The second extraction ensures quantitative recovery of the chemical from the plant material.

The solvents are distilled and recovered.

Repeated extractions of the tarry residues with boiling hot distilled water, in which the nordihydroguaiaretic acid is sparingly soluble, separate the chemical quantitatively from the tarry or resinous residues. The pH of the distilled water used was about 4.5, and there never was the slightest evidence of oxidation of the impure nordihydroguaiaretic acid with the boiling distilled water.

In obtaining 235.3 grams of crude nordihydroguaiaretic acid from 10,982 grams of the fresh, green machine-threshed creosote bush (Table I), 76 extractions of the tarry residues were made, each with 3 liters of boiling hot distilled water. The tar, after the solvents had been distilled off ("First Solvent Tank", Table I), was extracted 55 times, yielding 167.0 grams (1.52%) of the crude nordihydroguaiaretic acid, melting point 176–180° C. The last four or five extractions yielded virtually none, giving proof that the extraction was complete and quantitative. The tar from the "Second Solvent Tank," Table I, was extracted fifteen times, yielding 36.2 grams (0.33%) of the crude material, melting point 175–179° C. The last five extractions of this tar yielded no nordihydroguaiaretic acid, and were thus superfluous, except to ensure quantitative recovery.

In obtaining the yields of the crude material, the crude crystalline chemical was collected by filtering the cool aqueous solutions using filter cloths, then dried to constant weight and weighed.

The nordihydroguaiaretic acid (both colloidal and dissolved) was recovered from the combined aqueous filtrate liquors by extraction with sodium bisulfite–washed isopropyl ether; 5-gallon bottles were employed for separatory funnels. In each extraction, over 4 gallons of the aqueous filtrate liquor were shaken thoroughly with 1.5 to 2.0 liters of the washed isopropyl ether. This quantity of isopropyl ether was used for two or three extractions. The spent, extracted aqueous solution gave no red color with strong aqueous sodium hydroxide solution, thus indicating the absence of nordihydroguaiaretic acid and some other phenols from the extracted aqueous solutions. The extraction of the aqueous filtrate liquors with isopropyl ether has only analytical significance.

The isopropyl ether, used to extract the aqueous filtrate liquors, is distilled and recovered. The resulting tarry residue is extracted with boiling distilled water until the last one or two extractions (3 liters of boiling water per extraction) yield no crude nordihydroguaiaretic acid. Obviously these water extractions dissolve more of the impurities associated with the nordihydroguaiaretic acid, because the impurities were concentrated by means of extractions with the isopropyl ether.

A summary of the results obtained by extractions of the creosote bush samples, employing quantitative technique in the recoveries of the crude material, is shown in Table I.

All melting points were taken with a calibrated Fisher-Johns melting point apparatus.

The yield of crude nordihydroguaiaretic acid from the old, dry, hand-threshed sample is lower than from the fresh, green, machine-threshed samples.

PURITY OF CRUDE NORDIHYDROGUAIARETIC ACID

The product, which is quantitatively separated from the tar by means of boiling water, and having melting points noted in Table I, is termed crude nordihydroguaiaretic acid.

Acetylation of a sample of the crude material, melting point 177–181° C., with a considerable excess of acetyl chloride, produced a weight of acetyl derivative including the nordihydro-

Table II. Purification of Crude Nordihydroguaiaretic Acid

Starting Quantity Crude NDGA, M.P. 176–180° C., G.	Crystallization Solvent	Yield of Recrystallized NDGA, G.	Melting Point Recrystallized NDGA, ° C.
10.0	2.5% phenol	2.7	184.5–186
5.0	0.9% *n*-amyl alcohol	1.8	185–186
10.0	1.0% redistilled *n*-butyl alcohol	2.5	185–186
10.0	5.5% *n*-propyl alcohol	1.8	184–186
125.0	Redistilled 95% ethyl alcohol	67.6	183–185

guaiaretic acid tetraacetate, which indicated an apparent sample purity of 99.5%. This value undoubtedly is high, probably because of the presence, as impurities, of small amounts of other acetylatable chemicals, such as catechol, tannins, and flavanol or isoflavanol pigments (*17*).

Waller (*37*) observed that a 1% solution of bromine in chloroform reacted with nordihydroguaiaretic acid, with the evolution of hydrogen bromide gas and the formation of a brick-red bromination product. Attempts were made to adapt the U. S. Pharmacopoeia phenol assay procedure (*36*), which employs the 0.1 *N* bromine or Koppeschaar's solution, for quantitative determination. In this assay procedure (*36*), an excess of bromine quantitatively converts the phenol to the 2,4,6-tribromophenol. However, the reaction between the nordihydroguaiaretic acid and the bromine is not stoichiometric.

Working with samples of crude nordihydroguaiaretic acid, melting point 176–180° C., spectrophotometric data were obtained using the Lundberg and Halvorson (*21*) modification of the Emmerie and Engel (*7*) iron bipyridine method, which indicated an apparent sample purity of 92%. Although, as Lundberg and Halvorson (*21*) state, the reaction is almost completely lacking in specificity, the method described by these authors gives data which are a useful indication of the apparent purity of the sample in terms of nordihydroguaiaretic acid present. Samples available in 1945 melted at 177–180° C.

A spectrophotometric method has been reported (*6*).

PURIFICATION OF NORDIHYDROGUAIARETIC ACID

The crude nordihydroguaiaretic acid may be purified by simple recrystallization from the following hot solvents or solvent mixtures: redistilled 95% ethyl alcohol, *n*-butyl alcohol, *n*-butyl alcohol–water, glycerol-water, *tert*-butyl alcohol–water, phenol-water, *n*-amyl alcohol–water, *n*-propyl alcohol–water, and acetic acid–water. In most cases, the hot solution was filtered through cotton or paper before being cooled, with resulting separation of the purified nordihydroguaiaretic acid.

The nordihydroguaiaretic acid, melting point 184–185° C., may be obtained also by forming the tetraacetate from the crude material with acetyl chloride, separating the reaction by-products, then purifying an alcoholic solution of the tetraacetate ester with activated carbon, and, finally, hydrolyzing the tetraacetate with 2% hydrochloric acid and ethyl alcohol. The method of hydrolysis is described by Waller (*37*).

In Table II are shown some of the results obtained in the purification of the crude nordihydroguaiaretic acid by recrystallization from various solvents.

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Corrections

In the article on "Estimation of Amino Acids and Amines on Paper Chromatograms" [ANAL. CHEM., **22**, 1327 (1950)] "micromoles" should have been used in place of "millimoles" in several instances.

Page 1327, second column, third line under heading "Preparation of Standard Solutions."
Page 1328, Figure 2.
Page 1331, second column, fifth line under Table III.

RICHARD J. BLOCK

In the article "Analytical Applications of Ion Exchange Separations" [ANAL. CHEM., **22**, 1368 (1950)] reference to a personal communication from Henry Freiser was inadvertently omitted in connection with the following statement: "The stoichiometric release of hydrogen ions by the organic cation exchangers suggests a simple rapid means of preparing standard acids and bases."

JACK SCHUBERT

Spectrophotometric Study of the Platinum(IV)-Tin(II) Chloride System

GILBERT H. AYRES AND ALBERT S. MEYER, JR.
The University of Texas, Austin, Tex.

This paper is part of a general study of the analytical reactions of the platinum metals. Although titrimetric and colorimetric methods have been reported for the determination of platinum, the only generally accepted methods now in use are gravimetric. In addition to their usual limitations, the existing methods have little selectivity and require extensive separations prior to their application. The yellow color developed by the reaction of tin(II) chloride and platinum(IV) chloride in hydrochloric acid solution was studied both in the original aqueous solution and in extractions by organic solvents. The aqueous solutions were found to be stable, reproducible to ±0.1%, and, over a reasonable range, independent of reagent concentration. For the method used, the optimum concentration is from 3 to 25 p.p.m. of platinum. Over this range the minimum relative analysis error is less than 1%. By measuring solutions containing 50 to 60 p.p.m. of platinum against a 50 p.p.m. standard, the analysis error may be reduced to 0.1%. The color is almost quantitatively extracted into amyl acetate, and has essentially identical spectral characteristics as in the aqueous solution. The color fades rapidly, but may be stabilized for at least an hour by addition of 1% resorcinol. The extracted colors are reproducible only to about ±0.5% absolute transmittancy. A study was made of possible interference by many cations and common reagent anions; in general, the spectrophotometric method shows greater tolerance than gravimetric methods. The method, without loss of accuracy, saves time in determination of platinum and permits use of less rigorous preliminary separations; it is applicable to low concentrations.

ONLY two colorimetric methods for platinum have found much application; the tin(II) chloride method is superior to the potassium iodide method because of more rapid color development and greater selectivity (*7, 11, 13*).

The reaction of tin(II) chloride with platinum(IV) was first reported by Wöhler (*15*), who found that platinum(IV) in hydrochloric acid solution, when treated with tin(II) chloride gave a blood-red color that was extractable with ether. He considered the color to be due to colloidal platinum, analogous to the "purple of Cassius" formed when gold chloride is similarly treated (*16*). Later authors have attributed the color to platinum(II) (*6*), or to chloroplatinous acid (*12, 14*). The method has been applied to the estimation of small amounts of platinum filtered from air in the vicinity of platinum works (*6*), and to the determination of small amounts of platinum in nitric acid (*2*). Poluektov and Spivak (*10*) determined platinum in ores containing 0.03 to 0.1 gram of platinum per ton; the developed color was extracted into ethyl acetate. Sandell (*11*) studied the method photoelectrically, using a blue filter, and showed that the system conformed to Beer's law in the concentration range investigated (up to 2 p.p.m.); he found strong interference from palladium, and lesser interference from ruthenium, gold, and iron. Wölbling (*17*) found that if the solution was first made ammoniacal, and then acidified to about 1 molar before addition of tin(II) chloride, the palladium color was not extracted, while the platinum color was not appreciably affected. Hopkins (*5*) states that the tin(II) chloride test for platinum must be carried out in the absence of organic matter; the original reference (*8*) indicates that interference is due to the yellow color formed when the sample is treated with aqua regia, rather than to the organic matter per se.

It was the purpose of the present investigation to make a detailed spectrophotometric study of the platinum(IV)-tin(II) chloride color system, both in aqueous solution and in organic solvent extracts; to establish optimum conditions for color formation; to evaluate the photometric accuracy; to determine the nature and extent of interferences, and methods for their elimination; and to attempt to elucidate the chemistry of the color-forming reaction.

APPARATUS

Transmittancy measurements were made with a Beckman Model DU spectrophotometer, using matched 1.000-cm. cells. The instrument was operated at constant sensitivity, using slit widths of the order of 0.02 to 0.1 mm., corresponding to nominal band widths of about 1 to 4 millimicrons.

For preparation and gravimetric analysis of the standard platinum solution, weighings were made on an assay balance having a sensitivity of 0.002 mg., using weights that had been calibrated directly against National Bureau of Standards certified weights. Calibrated volumetric ware was used throughout.

REAGENTS

Grade 1 platinum thermocouple wire, specified as 99.99% pure, was used for preparation of the standard platinum solution.

Tin(II) chloride solution, 1.0 molar in tin(II) chloride and 3.5 molar in hydrochloric acid, was prepared from AMERICAN CHEMICAL SOCIETY reagent grade tin(II) chloride dihydrate. After the salt was dissolved in hydrochloric acid and diluted to volume, the clear solution was separated by decantation from the small amount of residue, and stored under a layer of xylene to protect against atmospheric oxidation.

Stock solutions used for the study of interfering metals contained 1 mg. of metal per ml. All chemicals were A.C.S. reagent grade, except compounds of the platinum metals, which were Eimer and Amend C.P. materials. Solutions of copper(II), cobalt(II), nickel(II), chromium(III), palladium(II), rhodium(III), iridium(IV), and ruthenium(III) were prepared from their chlorides. Iron(II) solution was prepared from ferrous ammonium sulfate hexahydrate; tellurium(IV) was prepared by dissolving the dioxide in sulfuric acid. Gold(III) solution was made from chloroauric acid monohydrate. Osmium(IV), as chloroosmate, was prepared from the pure tetroxide. For testing anion interference, chloride, bromide, and sulfate were used in the form of their alkali salts; because of the high concentrations necessary to show any interference, these were added in the form of weighed amounts of the solid salts. Perchlorate was added in the form of accurately measured volumes of perchloric acid.

Baker and Adamson purified isoamyl acetate was used for the extractions; 1% resorcinol was added to this solvent, to stabilize the extracted color.

EXPERIMENTAL

Preparation of Standard Platinum Solution. Exactly 1 gram of grade 1 platinum thermocouple wire (99.99% pure) was dissolved in aqua regia, evaporated almost to dryness, taken up with 20 ml. of 1 to 1 hydrochloric acid, and again evaporated to sirupy consistency. The hydrochloric acid treatment was repeated three times to remove all nitric acid and to destroy any nitrosoplatinic acid. After final evaporation, the material was transferred to a 1-liter volumetric flask, 10 ml. of concentrated hydrochloric acid were added, and the solution was diluted to volume, giving a concentration of 1 mg. of platinum per ml.

The concentration of the solution was checked by precipitating the platinum from 25-ml. aliquots with formic acid, finally igniting and weighing as platinum (*3*, p. 290). The results of triplicate analyses were as follows:

Sample No.	1	2	3	Mean
Platinum found, mg.	25.07	25.16	25.20	25.14
Deviation, %	0.28	0.08	0.24	0.20

The slightly high results are believed to be due to silica, because the flask in which the platinum was dissolved was slightly etched.

Color Development and Measurement. The desired volume of standard platinum solution was transferred to a 100-ml. volumetric flask, 10 ml. of concentrated hydrochloric acid, 25 ml. of 20% ammonium chloride solution, and 20 ml. of 1.0 molar tin(II) chloride solution were added, and the mixture was diluted to volume. A blank was prepared from identical amounts of reagents. A portion of the developed solution was used for transmittancy measurements. Another portion was extracted with an equal volume of amyl acetate; an amyl acetate blank was prepared by extracting the aqueous blank.

The aqueous solutions had a transmittancy of approximately 100% in the range of 1000 to 650 mμ; a minimum transmittancy was found at 403 mμ, and a sharp maximum at 355 mμ. A second minimum was located at about 310 mμ, but below 325 mμ the absorption of the blank was so great as to render this minimum unsuitable for use. The spectral characteristics of the amyl acetate extract were almost identical with those of the aqueous solution, except that the minimum was displaced to 398 mμ. Curves of transmittancy versus wave length for the aqueous solutions of various concentrations are shown in Figure 1.

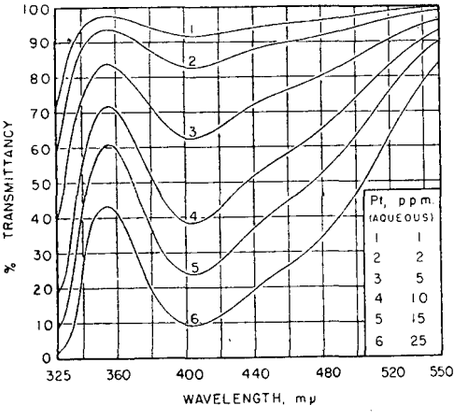

Figure 1

Stability of Color. The color developed so rapidly in the aqueous solution that it was impossible to obtain readings before complete development was attained. To test the stability of the color, solutions containing 1, 10, and 30 p.p.m. (mg. per liter) of platinum were developed by the above procedure; these concentrations more than covered the optimum concentration range. Transmittancy measurements at 403 mμ were taken immediately and at intervals over a period of 5 days. The aqueous solutions showed no change in transmittancy over this period. Upon storage for several weeks, considerable fading occurred; apparently the fading was caused by atmospheric oxidation, for the addition of more tin(II) chloride restored the color. Although the color in amyl acetate appeared to be stable while in contact with the aqueous solution, after separation of the phases was made it faded too rapidly for measurement. The color in ethyl acetate was somewhat more stable than in amyl acetate, but even this showed rapid fading. This was found to be due to atmospheric oxidation of the tin(II) chloride, and was more rapid in amyl acetate than in ethyl acetate because of the lower solubility of tin(II) chloride in the former.

Various inorganic and organic reducing agents were added, both before and after extraction, to test for stabilizing effect. These included sulfurous acid, crystals of tin(II) chloride, hydroxylamine hydrochloride, formic acid, oxalic acid, and resorcinol. Sulfurous acid formed a pale yellow precipitate in the organic layer. All the other reagents gave some increase in color stability, but only resorcinol gave a stability suitable for quantitative measurement of transmittancy. When amyl acetate containing 1% resorcinol was used as the extractant, the solutions were found to give constant transmittancy readings for at least an hour, the color stability increasing as the platinum concentration decreased.

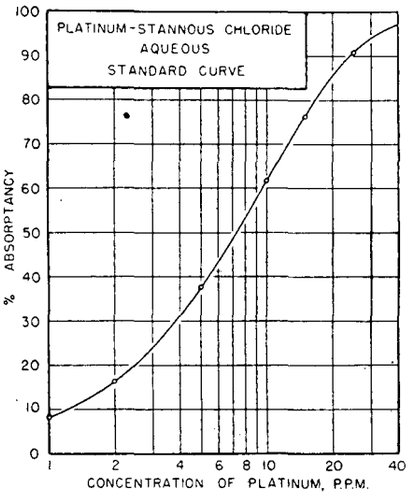

Figure 2

Optimum Reagent Concentrations. Using a constant amount of platinum (10 p.p.m. in the final solution, which is about in the middle of the optimum concentration range), solutions were developed with reagent concentrations varying within wide limits. Transmittancies of both the aqueous and the extracted solutions were measured over the range 450 to 345 mμ (to detect any possible shift in the minimum). The hydrochloric acid produced little change in the transmittancy, provided that the amount added was greater than about 6 ml. of concentrated acid per 100 ml. of final solution. Below this amount, with decreasing hydrochloric acid concentration the transmittancy of the aqueous solution decreased rapidly and that of the organic layer increased somewhat less rapidly. Tin(II) chloride reagent used in amounts from 5 to 30 ml. gave nearly constant transmittancies in both phases. If palladium is present and 10 ml. of concentrated hydrochloric acid are used, at least 20 ml. of tin(II) chloride should be added to prevent extraction of the palladium color into the organic solvent. With increasing concentration of hydrochloric acid, larger amounts of tin(II) chloride are necessary to prevent palladium interference. Ammonium chloride was added to prevent cloudiness in the extracted layers; 25 ml. of 20% solution were adequate. The transmittancy of the solutions was not influenced by the addition of ammonium chloride up to 30 ml.

On the basis of the results of these tests, the procedure described under "Color Development" was adopted; the final solution was about 2 molar in hydrochloric acid, 1 molar in ammonium chloride, and 0.2 molar in tin(II) chloride. The transmittancy of a portion of the aqueous solution was measured. A measured volume of the aqueous solution was extracted with an equal volume of amyl acetate (containing 1% resorcinol) by shaking for 1 minute; the organic layer was separated and dried for 5 minutes over silica gel, and its transmittancy was measured, the blank consisting of a similar extract of the aqueous blank. Both the aqueous and the organic solutions showed a small increase in

transmittancy with increase in temperature; hence they were allowed to stand in the spectrophotometer for 10 minutes to reach thermal equilibrium before transmittancy readings were made. Stable readings were obtained after this equilibration period.

Reproducibility. Samples of the same concentration gave transmittancy readings, on the aqueous solution, which seldom differed by more than 0.1%. To attain this precision, it was found advisable to clean the absorption cells with chromic acid cleaning mixture at least daily; a 1-minute treatment with the cold cleaning mixture was satisfactory. Solutions heated to boiling and cooled after color development but before dilution to final volume were reproducible to 0.1% transmittancy. However, if the solutions were boiled for several minutes, the final solutions showed a decrease of about 1% absolute transmittancy.

Leutwein (*9*) has shown that dilute solutions of the platinum metals stored in glass bottles underwent a significant change in concentration. This effect was confirmed by the authors; dilute platinum solutions (0.001 *M*) which had been stored for several weeks (in borosilicate glass-stoppered bottles), then color developed by the standardized procedure, had transmittancies corresponding to 1 to 2% relative decrease in platinum concentration. For this reason, solutions freshly diluted from the stock standard solution were used for calibration data.

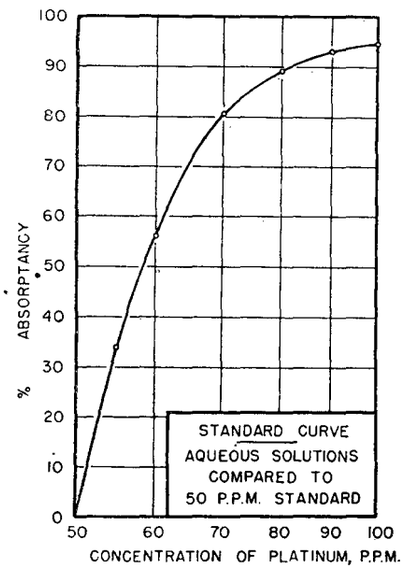

Figure 3

With the organic extracts, the most careful attempts to reproduce conditions indicated larger indeterminate errors than for the aqueous solutions; the reproducibility for the extracts was about 0.5% absolute transmittancy. Larger errors may be introduced through instability of the resorcinol in the amyl acetate extractant. When freshly prepared the solution was pale yellow, and gradually changed to a red tint on standing; once started, this change accelerated rapidly, and serious errors resulted if the mixture was prepared more than one week in advance. By simultaneous preparation of samples and blank with fresh solution, no interference from this source was encountered.

Effect of Temperature. The transmittancy of a color-developed sample containing 10 p.p.m. of platinum was measured over the range 20° to 50° C. With increasing temperature, the transmittancy increased at a rate of 0.07% absolute transmittancy per 1° C.; the effect was completely reversible.

Effect of Platinum Concentration. Solutions containing a final concentration of platinum from 1 to 25 p.p.m., in suitable increments, were developed by the standardized procedure, and the transmittancies of both the aqueous solution and the organic extract were measured over the range of 325 to 650 mμ. Minimum transmittancy occurred at 403 mμ for the aqueous solutions, and at 398 mμ for the amyl acetate solutions. The transmittancies at these wave lengths are shown in Table I. The data for the aqueous solutions are shown in Figure 2, in which per cent absorptancy (100 − % transmittancy) is plotted against log concentration.

Table I. Transmittancies of Platinum(IV)-Tin(II) Chloride Solutions

Concentration of platinum, p.p.m.	1	2	5	10	15	25
% transmittancy of aqueous solution at 403 mμ	91.7	82.7	62.3	38.3	23.8	9.2
% transmittancy of amyl acetate solution at 398 mμ	92.8	84.3	64.4	39.5	24.2	9.7

The concentration can be extended to higher values, with an increase in accuracy, by the differential method (*1*, *4*), in which the transmittance of a sample is compared with the transmittance of a reference standard of slightly lower concentration instead of the customary blank solution. Aqueous solutions containing 55 to 100 p.p.m. of platinum were compared against a reference standard containing 50 p.p.m. of platinum, the measurements being made over a wave-length range around 403 mμ. The plot of per cent absorptance (at 403 mμ) against log concentration is shown in Figure 3. The transmittance ratios showed deviation from Beer's law when the sample solution was more concentrated than about 70 p.p.m.; above this concentration the position of minimum transmittancy shifted slightly in the direction of longer wave lengths.

Extraction Efficiency. In order to determine the extraction efficiency, color-developed samples containing 100 and 250 p.p.m. of platinum were prepared. One portion of each solution was extracted with an equal volume of amyl acetate; another portion of each solution was extracted with only one fifth of its own volume of amyl acetate. The platinum remaining in the aqueous layer was determined spectrophotometrically at 403 mμ; the color density in the organic solvent was too great for direct measurement, and was obtained by difference. The extraction coefficient was calculated on the assumption that no volume changes occurred in either of the phases. The results are shown in Table II.

Table II. Extraction Efficiency

Original concentration of platinum (aqueous), p.p.m.	100	100	250	250
Aqueous solution used, ml.	25	50	25	50
Amyl acetate used, ml.	25	10	25	10
Transmittancy of aqueous layer, %	96.8	84.1	92.5	67.2
Concentration of platinum in aqueous layer, p.p.m.	0.4	1.9	0.8	4.2
Concentration of platinum in amyl acetate layer (calcd.), p.p.m.	99.6	490	249	1230
Ratio of platinum concentration in aqueous layer–amyl acetate layer	0.0040	0.0039	0.0032	0.0034
Platinum extracted from equal volumes, %	99.6	99.6	99.7	99.7

The following experiments were performed to test the possibility of increasing the flexibility of the method by extracting the color from large volumes of aqueous solution into relatively small volumes of organic solvent, so that the method would be applicable to determination of minute absolute amounts of platinum.

A 10-ml. portion of solution containing 10 p.p.m. of platinum was extracted with 10 ml. of amyl acetate, and the transmittancy of the extract was measured. Another 10-ml. portion of the same solution was diluted to 50 ml. with the blank solution, and the diluted solution was extracted with 10 ml. of amyl acetate; a

similar blank was prepared simultaneously; the transmittancy of the organic extract was measured. The above process was repeated, using aqueous-organic ratios of 10 to 1. The transmittancies at 398 mμ are shown in Table III.

Table III. Concentration by Extraction

	Ratio of Aqueous Volume to Organic Volume				
In test solution	1:1	5:1	5:1	10:1	10:1
In blank	1:1	1:1	5:1	5:1	10:1
Transmittancy of organic extract, %	37.7	34.2	35.8	33.0	33.4

Effect of Diverse Ions. The following were the observed reactions of the platinum metals when a test solution containing 1 mg. of metal was treated with 5 ml. of concentrated hydrochloric acid and 5 ml. of tin(II) chloride and diluted to 25 ml., giving a final concentration of 40 p.p.m. of metal.

PLATINUM. The test solution was very pale yellow; on addition of tin(II) chloride an intense yellow-orange color developed, reaching its maximum intensity with less than 1 ml. of the reagent. The colored material was completely extractible into ether, ethyl acetate, or amyl acetate. Aqueous and organic solutions left in contact showed no apparent change over 12 days. Addition of 5 grams of ammonium chloride before the addition of tin(II) produced no immediate precipitation of ammonium chloroplatinate even in solutions containing up to 200 p.p.m. of platinum; solutions containing 20 p.p.m. gave a slight precipitate after 24 to 48 hours; the precipitate dissolved slowly after the addition of tin(II) chloride.

OSMIUM. The test solution was amber; no change was observed on adding the reagents. There was no evidence of extraction.

IRIDIUM. The test solution was orange; on addition of the tin(II) chloride, the color changed to pale yellow. Part of the colored material was extractable, giving a pale yellow color to the organic solvent. The solutions were stable for at least 12 hours.

RUTHENIUM. On addition of tin(II) chloride to the dark orange solution, the color changed to pale blue. Within about 12 hours the color had changed to pale yellow. There was no evidence of extraction into ether, ethyl acetate, or amyl acetate.

RHODIUM. The test solution was light red in color. Addition of the reagents produced a light yellow color. On standing, the solution slowly turned to a deep raspberry red. Both the yellow and the red solutions extracted to give a pale yellowish green solution in ether or the esters. The extracted solution was stable for at least 12 hours. No apparent differences were produced by adding ammonium chloride.

PALLADIUM. The test solution was light orange. An intense red-orange color developed with the first few drops of tin(II) chloride; on further addition of the reagent, the color changed to yellowish green, then to nearly black, and finally to dark olive green. The color partly extracted into ether or the acetates to give a red color to the organic layer and leave a green aqueous layer. Both colors were unstable, the organic solution turning to a lighter color and the aqueous solution turning reddish brown within 12 hours. The addition of ammonium chloride was without apparent effect.

A further study showed that the amount of palladium extracted depended upon both the concentration of hydrochloric acid and the concentration of tin(II) chloride. Using the same amount of palladium as before, but only one fourth as much hydrochloric acid, addition of tin(II) gave an intense yellow color, a large amount of which was extractable. As more tin(II) reagent was added, the color changed to emerald green, then to dark blue green, and finally to olive green; further addition of tin(II) chloride produced no further changes. The final olive-green material was extractable only to a slight extent. This material is possibly the colloidal metal; in a dialysis test there was no evidence that it passed through a collodion membrane.

Chloroform, carbon tetrachloride, and xylene were without effect in all the extraction tests.

The metallic ions selected for detailed study of interference were osmium(IV), iridium(IV), ruthenium(III), rhodium(III), palladium(II), gold(III), iron(II), cobalt(II), nickel(II), copper(II), chromium(III), and tellurium(IV). Anions included were bromide, sulfate, nitrate, and perchlorate. The diverse ions were added, individually, to solutions containing 10 p.p.m. of platinum (in the final solution), in amounts expected to give at least moderate interference; the color was developed in the usual way. The object of these tests was to determine the concentration of the added substance which would give interference corresponding to 1% relative error on the concentration of platinum. At this concentration a relative error of 1% corresponds to 0.4% absolute transmittancy; because the experimental error is relatively large with respect to 0.4% transmittancy, the diverse ions were added in amounts that would give absolute transmittancy changes of 1% or more. Readings were made on solutions of at least three concentrations of interfering ion, and the value for 0.4% absolute transmittancy difference was obtained graphically from a plot of the change in transmittancy against concentration of interfering agent. The interference graphs are shown in Figures 4 and 5. Copper, iron, cobalt, nickel, and all the anions gave relatively small interferences and were not plotted.

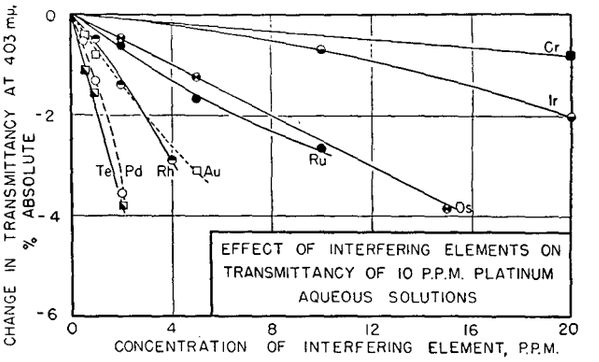

Figure 4

The platinum solutions to which other platinum metals were added underwent rather rapid initial changes in color after the addition of the tin(II) reagent; therefore, transmittancy measurements on the aqueous color were not made until equilibrium was attained—i.e., when there was no further change in transmittancy at 403 mμ. This usually required about 30 minutes, but in some cases required about an hour. Because of the rapid initial color change of the aqueous solution, a portion of the solution was extracted immediately, for measurement of the organic extract. In the case of gold, the interference in the extracted phase seemed to be due largely to coprecipitation of platinum; because this might change with time, portions of the platinum-gold mixture were extracted immediately. In all other cases of the diverse ions studied, the final aqueous colors were stable, hence were extracted when convenient, usually after about 4 hours.

The concentrations of the diverse ions required to produce a

Table IV. Effect of Diverse Ions

(All solutions contained 10 p.p.m. of platinum)

	Aqueous Solution		Organic Extract	
Interfering Substance	Concn., p.p.m., for 1% relative error	% relative to platinum	Concn., p.p.m., for 1% relative error	% relative to platinum
Osmium(IV)	1.5	15	>50	>500
Iridium(IV)	4.8	48	11	110
Ruthenium(III)	1.4	14	40	400
Rhodium(III)	0.8	8	0.1	1
Palladium(II)	0.4	4	2.3	23
Gold(III)[a]	0.5	5	0.7	7
Tellurium(IV)[a]	0.3	3	0.1	1
Chromium(III)	10	100	90	900
Nickel(II)	40	400	>200	
Iron(II)	2×10^3		2×10^3	
Cobalt(II)	2×10^3		2×10^3	
Copper(II)	2×10^3		2×10^3	
Nitrate	$>6 \times 10^4$		$>2 \times 10^4$	
Perchlorate	$>2 \times 10^4$			
Sulfate	$>2 \times 10^4$			
Bromide	$>2 \times 10^4$			

[a] All substances produced a decrease in transmittancy, except that in only the extracted phase gold and tellurium produced an increase.

transmittancy difference corresponding to 1% relative error in measuring 10 p.p.m. of platinum are shown in Table IV.

DISCUSSION

The platinum(IV)-tin(II) chloride color system conforms to Beer's law up to a concentration of at least 30 p.p.m. of platinum when samples are measured against a reagent blank. In the differential method, using 50 p.p.m. as the reference standard, the system follows Beer's law up to about 70 p.p.m. Deviations from the law at higher concentrations appear to be due at least in part to a shift in the position of minimum transmittancy toward longer wave lengths. In the calibration curve, Figure 2, per cent absorptancy (100 − % transmittancy) at 403 mμ for the aqueous solutions is plotted against log concentration. Reference to Table I shows that a similar plot of the organic extracts would be almost coincident with the curve of Figure 2; hence the following considerations would apply equally well to either phase. The inflection point in the curve occurs at 63% absorptancy, and the slope of the curve at this point corresponds to a relative error of 2.7% per 1% absolute photometric error, in conformity with derivations from Beer's law (*1*). For a precision of 0.2% absolute transmittancy (aqueous solutions), the minimum relative error is therefore 0.5%; a precision of 0.5% absolute transmittancy in measuring the organic extracts corresponds to a minimum relative error of 1.4%. Minimum error occurs at about 10 p.p.m. of platinum, although the error is not appreciably greater in the range of 5 to 20 p.p.m.; in order to keep the relative error within 1%, for aqueous solutions the concentration range must be within the limits 3 to 25 p.p.m. of platinum.

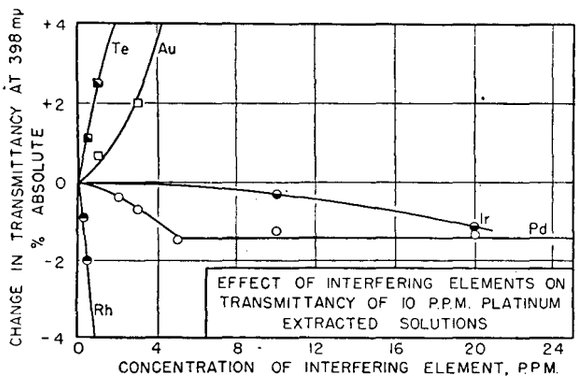

Figure 5

By the differential method (measurement against a reference standard instead of a blank), the range can be extended to higher concentrations; by this method the accuracy can be increased (*1*). From Figure 3 it can be shown that at 60 p.p.m. the relative error is only 0.5% per 1% absolute photometric error, or a relative error of 0.1% for a precision of 0.2% absolute in reading the transmittancy; at about 85 p.p.m., the relative error is about 2.7% per 1% absolute photometric error, which is the same as the accuracy attainable in the range of 5 to 20 p.p.m. when samples are measured against a blank solution. The range could not be extended to much higher concentrations, inasmuch as 50 p.p.m. of platinum is approaching the limit of color density of a reference standard which can be balanced in the instrument used. No attempt was made to apply the differential method to organic extracts, because the stability of the extracted solutions decreased with increasing platinum concentration.

Although in general the aqueous solutions proved to be superior to the extracted solutions for measurement, the extraction procedure provides a method of concentrating the material being determined. The high distribution coefficient of the colored system between organic solvent and aqueous solution enhances the applicability of this method. A large number of factors may influence the intensity of the extracted color and cause transmittancy variations when the relative volumes of the two phases are varied; two factors of considerable importance are the distribution coefficients of colored species and of reagents, and the mutual solubilities of the two phases—i.e., changes in volumes of the layers on mixing. The reagent concentrations in the aqueous phase were selected on two criteria: first, that slight changes in reagent concentrations produce little change in transmittancy; and second, when possible the reagent concentrations were made high to decrease the solubility of the organic solvent and to minimize the reagent distribution effects. It is possible, of course, to correct for volume changes in the organic layer by carefully separating the two phases and diluting the organic solution to definite volume; this would require additional time, and when the extraction is used, to concentrate the color into a small volume would be impractical.

At the reagent concentrations selected, the transmittancies of the developed solutions (either aqueous or organic phase) are not sensitive to small changes in amounts of reagents; errors of as much as 1 ml. in measuring the hydrochloric acid, tin(II) chloride, or ammonium chloride were without effect on the transmittancy. The data of Table III show the effect of changing the ratio of aqueous to organic phase on the transmittancy of the organic extract; both the sample and the blank were affected. These results indicate that a separate standard curve is required for each volume ratio that would be used; otherwise considerable error would be introduced. In cases where only a small amount of platinum is present, or the volume of the solution is large, the extraction method may prove advantageous. Approximately 3 ml. of solution are required to fill the absorption cells used; the colored material may therefore be extracted with as little as 5 ml. of amyl acetate. From a volume of 25 ml. of aqueous solution, the platinum can be concentrated by a factor of five by such an extraction.

The interference by palladium in the amyl acetate extract is unique, in that interference increased up to a concentration of about 5 p.p.m., after which it remained constant up to concentrations of 50 p.p.m. or greater. For determination of platinum in the presence of small amounts of palladium, interference from the latter could be prevented by the use of a calibration curve for platinum solutions to which palladium had been added in an amount greater than 5 p.p.m.; addition of at least 5 p.p.m. of palladium to the unknown platinum sample would ensure measurements on the plateau of the palladium interference, and reference to the special calibration curve would cancel the effect of the palladium. A simpler alternative would be the addition of at least 5 p.p.m. of palladium to both the sample and the blank solution. These procedures would be subject to some error, because the extracted color of palladium is very sensitive to reagent concentrations.

Table IV shows that the extracted solutions have more selectivity than the aqueous solutions, for the amount of interfering substance to produce a given relative error. With the exception of rhodium, and to a degree tellurium, extraction increased the tolerance of the platinum system for the other substances. It is unfortunate that the greatest improvement by extraction is for those substances which are most easily separated from platinum—namely, osmium and ruthenium; these elements are separated, by volatilization as their tetroxides, from the other platinum metals. For platinum determination in the presence of rhodium, however, use of the aqueous solution rather than the organic extract is indicated; this would probably be the case even in the presence of palladium, because palladium is more easily separated from platinum than is rhodium.

The tolerances for palladium, rhodium, gold, and tellurium leave much to be desired. However, for a given relative error in the platinum determination, the spectrophotometric method

does not require as complete a separation from these elements as is necessary for gravimetric determination. The spectrophotometric method offers an especially advantageous means for determining platinum in the presence of the more base metals of the acid hydrogen sulfide group; determination of platinum through the sulfide (*3*, p. 289) requires absence of all other elements that are precipitated with hydrogen sulfide in acid solution.

Wöhler and Spengel (*16*) suggested that the color produced in the reaction of platinum(IV) with tin(II) chloride was due to colloidal platinum. This suggestion is probably erroneous, in view of the fact that the colored material readily passes through semipermeable membranes such as collodion. Further evidence that the colored species is not the colloidal metal is shown by the rapid and complete extraction into organic solvents. The color has been attributed also to platinum(II) (*6*). However, the authors found that when the platinum(IV) chloride solution (dilute chloroplatinic acid) was evaporated to fumes with sulfuric acid to expel hydrochloric acid, and the sulfuric acid solution was treated with tin(II) sulfate, no color developed. Simple reduction to platinum(II), therefore, cannot account for the color reaction. Furthermore, addition of hydrochloric acid to the colorless platinum and tin sulfate solution immediately produced an intense yellow color. It appears, therefore, that chloride ion is one of the requisites for color formation.

The color is not due to chloroplatinous acid, as has been claimed (*12*, *14*). A solution containing 10 p.p.m. of platinum as chloroplatinous acid (potassium chloroplatinite in 10% hydrochloric acid solution, prepared so as to prevent any possible atmospheric oxidation) was essentially colorless. Upon the addition of tin(II) chloride to this solution, an immediate yellow color developed, comparable in intensity with that produced from 10 p.p.m. of platinum(IV). A solution containing 1000 p.p.m. of platinum as chloroplatinous acid had a color intensity comparable to that produced by 10 p.p.m. of platinum(IV) in the tin(II) reaction. The spectral curve (transmittancy versus wave length) of the 1000 p.p.m. solution of chloroplatinous acid was of the same general shape, below 420 mμ, as the curve for the solutions from the platinum(IV)-tin(II) chloride reaction (Figure 1), except that the maximum occurred at 360 mμ (instead of 353 mμ), and the minimum occurred at 390 mμ (instead of 403 mμ); in addition, the chloroplatinous acid had another minimum at 475 mμ. It is obvious, therefore, that the color is not due simply to chloroplatinous acid, but in some way involves the tin. A further study of the chemistry of the reaction process is under way in this laboratory, and will be made the subject of a later report.

ACKNOWLEDGMENT

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Spectrophotometric Determination of Molybdenum with Phenylhydrazine Hydrochloride

GILBERT H. AYRES AND BARTHOLOMEW L. TUFFLY
The University of Texas, Austin, Tex.

THE most widely used photometric method for the determination of molybdenum is based upon the amber to orange color produced by treatment with thiocyanate and a reducing agent, usually tin(II) chloride; ordinarily, the colored complex is extracted into butyl acetate for the color measurement (*10*). The color intensity is dependent upon a number of variables (*6*); one of the serious disadvantages of the method is fading of the color, and the rather rigid control necessary to minimize this effect. By using water-acetone solutions and tin(II) chloride reduction, Grimaldi and Wells (*4*) eliminated the extraction procedure, and found the acetone to exert a stabilizing effect on the color. Recently, Ellis and Olson (*2*) used acetone as the reducing agent, and found that this increased both the color stability and the sensitivity; extraction into organic solvents was not recommended.

Among the several other color reactions of molybdenum that have been observed, it appeared that the reaction with phenylhydrazine might prove useful for photometric analysis. Spiegel and Maass (*9*) observed that molybdates in acid solution reacted with phenylhydrazine to produce a blood-red color or a red precipitate. The reaction was later studied by Montignie (*8*), and reported to be specific for molybdenum; color formation was said to involve the oxidation, by molybdate, of the phenylhydrazine to a diazonium salt, which then coupled with the excess phenylhydrazine and molybdate. The method has been used as a spot test for molybdenum (*3*), but appears not to have been studied for application to quantitative spectrophotometric analysis. It was the purpose of this investigation to study the molybdenum-phenylhydrazine reaction to establish the best con ditions for color development, to evaluate the optimum range and the accuracy of the photometric process, to determine the nature and extent of interference from diverse ions, and to test the applicability of the method to the spectrophotometric determination of molybdenum in steel.

This investigation was undertaken to study the red color system produced when acid solutions of molybdenum(VI) are treated with phenylhydrazine hydrochloride, with a view to its application to the spectrophotometric determination of molybdenum in steel. The red color is produced by treating molybdenum(VI) with an excess of phenylhydrazine hydrochloride, in 50% acetic acid solution. The color develops rapidly in hot solution, and is reproducible and stable. The optimum concentration range is from 2 to 10 p.p.m. Iron(III) may be tolerated up to a 20 to 1 weight ratio to molybdenum; for higher ratios, iron interference is eliminated by selective reduction. The usual alloying elements do not interfere. The method has been tested by analyzing Bureau of Standards steels, one of high and one of low molybdenum content. The method is simple, rapid, and accurate; it is applicable in steel analysis without change in usual dissolution procedures. It has the advantage over thiocyanate method that no extraction into organic solvents is involved and color is not subject to fading.

REAGENTS

Ammonium heptamolybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, was used for the preparation of standard molybdenum solution. Computed on the assay of 80.5% molybdenum trioxide (theoretical, 81.4% MoO_3), 9.315 grams of ammonium heptamolybdate tetrahydrate were dissolved in water containing 10 ml. of concentrated ammonium hydroxide and diluted to 500.0 ml.; the solution contained exactly 10 grams of molybdenum per liter. Working standards were prepared by quantitative dilution of the stock solution.

Eastman's phenylhydrazine hydrochloride was used in the final procedure; phenylhydrazine acetate and also the phenylhydrazine base were used in some of the preliminary testing.

Glacial acetic acid, and the salts used in the study of interferences, were analytical reagent grade.

National Bureau of Standards steels No. 153, 8.39% molybdenum, and No. 139, 0.178% molybdenum, were used in testing the application of the method.

APPARATUS

Transmittancy measurements were made with a Beckman Model DU spectrophotometer, using matched 1.000-cm. cells. The instrument was operated at constant sensitivity, using slit widths corresponding to band widths of about 2 to 4 millimicrons.

Calibrated weights and calibrated volumetric ware were used throughout.

EXPERIMENTAL

Spectral Characteristics. Solutions of several concentrations of molybdenum were color-developed with phenylhydrazine hydrochloride in acid solution at elevated temperature, and the transmittancy was measured at frequent wave-length intervals over the range 375 to 700 mμ. The curves of transmittancy versus wave length had a minimum at 505 mμ. This wave length was used for making transmittancy measurements in studying the influence of various factors on the color; a moderate wavelength region on either side of 505 mμ was scanned to detect any possible shift in the position of minimum transmittancy.

Figure 1 shows the spectral curves for solutions containing from 1 to 10 p.p.m. of molybdenum, developed by the standardized procedure described below. In the concentration range studied, the system conforms to Beer's law.

A concentration of 5 p.p.m. of molybdenum is about in the middle of the optimum concentration range; a constant amount of molybdenum, 5 p.p.m., was therefore used in testing the influence of various factors on the color intensity.

Molybdenum Oxidation State Required. Molybdate solutions which were reduced with tin(II) chloride gave no color when treated with the phenylhydrazine reagent. Oxidation state +6 (molybdate) is required for the color reaction.

Phenylhydrazine Reagent. Use of the phenylhydrazine base was not satisfactory on account of the ease with which the liquid oxidized to give yellow to brown colored oxidation products; photometric results were not precise when the free base was used. The same difficulty was experienced with a sample of phenylhydrazine acetate which contained some free base; the product recrystallized from ether gave a considerable improvement of precision. Satisfactory results were obtained with phenylhydrazine hydrochloride; hence this reagent was adopted for use. For work of high accuracy, it is advisable to use blanks prepared concurrently with samples; for routine work, blanks should be not more than 8 hours old.

Figure 2 shows the influence of varying amounts of phenylhydrazine hydrochloride (same amount in both sample and blank) on the transmittancy of solutions containing 5 p.p.m. of molybdenum; with 10 p.p.m., minimum transmittancy was reached also with 1 gram of reagent. In order to provide an adequate amount of reagent to ensure full development of amounts of molybdenum in the high portion of the working range, the use of 1.5 grams of phenylhydrazine hydrochloride per 100 ml. of final solution was adopted for the standard procedure. When 1.5 grams of phenylhydrazine hydrochloride were used for color development, the amount of the reagent in the blank could be varied within the limits of 1 to 2 grams without influencing the measured transmittancy.

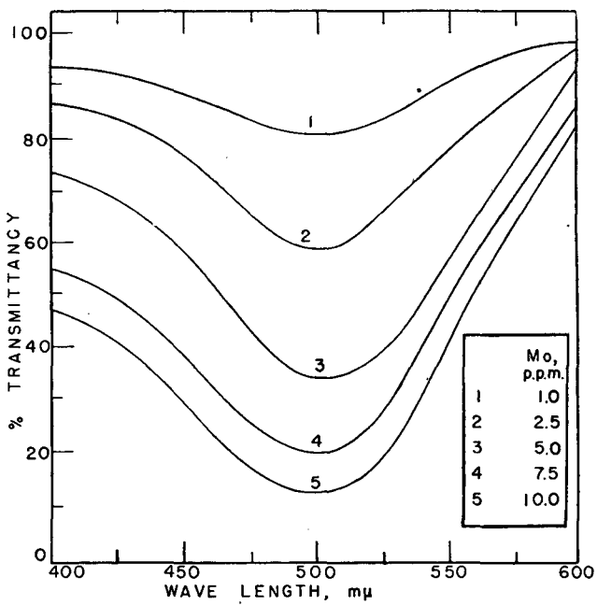

Figure 1. Spectral Curves for Molybdenum with Phenylhydrazine Hydrochloride

Effect of Acids. Reproducible results were not obtained with hydrochloric acid or sulfuric acid, and the effect of increasing the acid concentration was a decrease of color intensity. When acetic acid was used, it was found that increasing the acid concentration increased the color intensity—i.e., decreased the transmittancy—up to 50% acid (by volume), above which the

Table I. Rate of Color Development

(5 p.p.m. of molybdenum; 1.5 grams of phenylhydrazine hydrochloride per 100 ml. of 50% acetic acid; transmittancies at 505 mμ)

At Room Temperature		At Boiling Temperature	
Time, min.	% transmittancy	Time, min.	% transmittancy
0	99.8	0	99.8
10	78.0	1	36.8
20	61.3	2	35.5
30	55.3	3	35.0
60	47.7	4	35.0
100	45.2	5	35.0
150	42.3	10	34.9
200	39.2	15	35.0
250	37.5		
300	36.7		
350	35.2		
390	35.0		
450	35.0		

color intensity remained constant (Figure 3). Replicate samples gave good reproducibility.

Rate of Color Formation. The characteristic red color developed very slowly at room temperature, but formed very rapidly at the boiling point, as shown in Table I.

Stability of Color. Color-developed samples gave constant transmittancies over a period of 2 to 3 weeks, if compared with a blank of the same age. On longer standing, a dark scum separated in both blank and sample.

Temperature Coefficient of Transmittancy. Over the temperature range of 20° to 30° C. the transmittancy was constant; from 30° to 40° C. the transmittancy increased at an average rate of 0.08% (absolute) per 1° C.

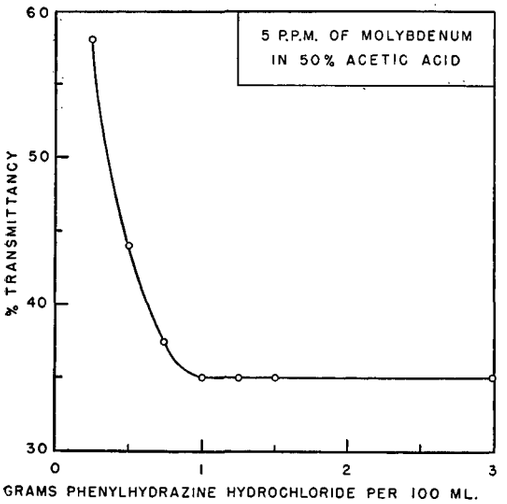

Figure 2. Effect of Phenylhydrazine Hydrochloride Concentration on Transmittancy at 505 mμ

Standardized Procedure. An aliquot of the working standard solution, or sample for analysis, to give the desired final concentration of molybdenum (optimum range, 2 to 10 p.p.m.) was added to 50 ml. of 50% acetic acid solution. After 1.5 grams of phenylhydrazine hydrochloride had been added, the mixture was boiled for 5 minutes. The cooled solution was transferred to a 100-ml. volumetric flask, 25 ml. of glacial acetic acid were added, and the solution was diluted to volume with water. Blank solutions prepared by the same method were usable for a period of about 8 hours. The transmittancy at 505 mμ was measured at room temperature (about 25° C.).

Reproducibility. In 24 replicate determinations, made over a period of several weeks, the transmittancy of solutions containing 5 p.p.m. of molybdenum averaged 35.0%, all results being within the limits 34.8 and 35.3%. The average deviation was 0.12%, and the standard deviation was 0.17% absolute transmittancy.

Effect of Diverse Ions. For application of the method to the analysis of molybdenum in steel, the possible interference from a number of metals must be considered. The other elements most commonly met in such samples are iron, chromium, manganese, nickel, cobalt, vanadium, and tungsten. For studying interference effects, these elements were used in the oxidation state that would be present in the solution after various dissolution procedures—namely, iron(III), chromium(III or VI), manganese(II), nickel(II), cobalt(II), vanadium(V), and tungsten(VI). By the use of phenylhydrazine hydrochloride as the color-developing reagent, some of the elements of high oxidation state are reduced—e.g., chromium(VI) to chromium(III).

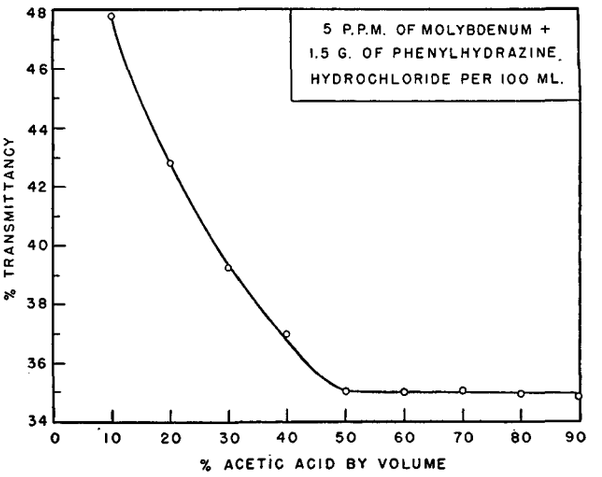

Figure 3. Effect of Acetic Acid Concentration on Transmittancy at 505 mμ

In order to establish the tolerance of the molybdenum system for another element, solutions containing 5 p.p.m. of molybdenum and added increments of the foreign element were developed and measured in the usual way. The tolerance was taken as the largest amount of foreign element that would give a transmittancy within 0.4% of that of the molybdenum alone. The tolerances are given in Table II.

Removal of Iron(III) Interference. Interference of iron(III) deserves special mention, because the principal application of the method would be the determination of molybdenum in steel. As shown in Table II, iron(III) does not interfere when the ratio of iron to molybdenum is less than 20 to 1; hence, for steels containing more than 5% molybdenum, removal of iron(III) is unnecessary. For low-molybdenum steels, however, removal of at least a considerable part of the iron(III) would be required. Because iron(II) can be tolerated in very large amounts, the obvious procedure is to reduce iron(III) to iron(II). However, the spectrophotometric determination requires molybdenum in the oxidation state +6. The problem, therefore, is to reduce the iron(III) without also reducing the molybdenum(VI). On the basis of the standard redox potentials of the systems involved (*7*), it would appear that the reduction of iron(III) to iron(II) with a Jones reductor, for example, would be difficult to accomplish without simultaneous reduction of molybdenum(VI) to molybdenum(III). On the other hand, if the reduction of iron(III) is very rapid in comparison with the rate of reduction of molybdenum(VI), it might still be possible to reduce moderate to large amounts of iron(III) without reducing small amounts of molybdenum(VI), by using a Jones reductor and a high flow rate. [Although reduction of molybdenum(VI) to molybdenum(III)

with a Jones reductor is a standard procedure for the titrimetric determination of molybdenum (*10*), the operating directions for this method do not specify any conditions of flow rate.]

For testing this procedure, a Jones reductor was prepared in a 50-ml. buret, using a 30-cm. column of amalgamated 20-mesh zinc. Samples of the molybdenum standard containing 0.5 mg. of molybdenum(VI) (to give a final concentration of 5 p.p.m. for spectrophotometric measurement) in 1 *M* hydrochloric acid were passed through the reductor at various flow rates, and the effluent solution was treated for color development by the standardized procedure. In a second series of experiments, the solutions contained 0.5 mg. of molybdenum(VI) and 250 mg. of iron(III) in 1 *M* hydrochloric acid (5 p.p.m. of molybdenum and 2500 p.p.m. of iron in the final solution for color measurement). After passing through the reductor, the molybdenum color was developed and measured. In a third series of tests, using molybdenum only, the solution from the reductor was delivered under ferric alum solution, and the iron(II) equivalent to the reduced molybdenum was titrated with 0.00250 *N* potassium permanganate solution.

The results of the reduction tests, summarized in Table III, show that when the flow rate is at least 0.5 ml. per second, interference from iron(III) can be eliminated in the spectrophotometric determination of molybdenum. The third experiment (ferric alum method) showed that at the high flow rates about 4% (0.02 mg.) of the molybdenum was reduced; because only the molybdenum(VI) develops the red color on treatment with phenylhydrazine hydrochloride, a reduction of 4% of the molybdenum present should be detectable in the spectrophotometric method as an absolute difference of about 1.5% transmittancy (see Discussion). The failure of the spectrophotometric measurement to show any reduction in these cases, and much less reduction at the slower flow rates, is due to the ease with which the reduced molybdenum is reoxidized at the boiling temperature during the color development procedure. This explanation was confirmed by using the effluent solutions from low flow rates, either molybdenum alone or molybdenum-iron mixtures, and subjecting them to a longer boiling period, and in some cases also aeration with a stream of compressed air. The color intensity increased, the transmittancy approaching 35.0%, the value for a fully developed sample of 5 p.p.m. of molybdenum.

In interpreting the results of the ferric alum titrimetric method, it should be noted that the sample contained only 0.5 mg. of molybdenum; although only about 50% of the molybdenum was reduced even by very slow passage through the reductor, it is clearly shown that at least with small amounts of molybdenum the amount of reduction is markedly influenced by the rate of flow of the solution through the reductor.

Table II. Effect of Foreign Elements

(All solutions, 5 p.p.m. of molybdenum)

Element Added	Tolerance, P.P.M.
Iron(III)	100
Iron(II)	$>3 \times 10^3$
Chromium(III)	100
Manganese(II)	$>5 \times 10^3$
Nickel(II)	$>5 \times 10^3$
Cobalt(II)	100
Vanadium(V), as metavanadate	4
Tungsten(VI)[a], as tungstate	20

[a] Tungsten produced an increase in transmittancy; all others gave a decrease in transmittancy.

Table III. Effect of Flow Rate through Jones Reductor

(All solutions, 0.5 mg. of molybdenum)

	Experiment 1, Molybdenum Only		Experiment 2, 250 Mg. of Iron(III) Added		Experiment 3, Molybdenum Only Ferric Alum Method,
Flow Rate, Ml./Sec.	% transmittancy	% Mo reduced	% transmittancy	% Mo reduced	% Mo Reduced
0.01	...	...	...	...	51
0.05	...	...	...	...	50
0.1	37.2	6.0	37.0	5.4	30
0.2	36.6	4.3	36.5	4.0	...
0.25	35.5	1.4	35.7	1.9	19
0.33	...	...	35.5	1.4	...
0.5	34.9	0	35.0	0	3.8
1.0	35.0	0	35.0	0	3.6

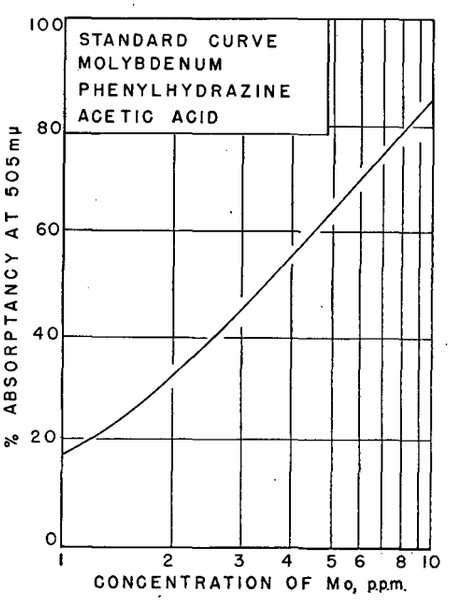

Figure 4. Calibration Curve

Iron(III) interference can be eliminated also by reduction with sulfur dioxide in acid solution; after excess sulfur dioxide has been boiled out, the color is developed in the usual way. In the authors' experience, this procedure is somewhat longer than the Jones reductor method. Removal of iron(III) by multiple precipitation of the hydrous oxide was too tedious to be practical, and the molybdenum found was always too low.

Assay of Molybdenum(VI) Oxide. An accurately weighed sample of reagent grade molybdenum(VI) oxide was dissolved in ammonium hydroxide and made to known volume. Several aliquots of the solution were developed by the standardized procedure, the transmittancy was measured, and the molybdenum concentration was read from the calibration curve. The molybdenum, calculated as MoO_3, was found to be 100%. The assay given by the manufacturer was 99.95% MoO_3.

Determination of Molybdenum in Steel. The National Bureau of Standards steels used, and the certified analyses for alloying metals, are shown in Table IV.

Table IV. Steels Tested

	Sample 139, Cr-Ni-Mo Steel (N.E. 8637), %	Sample 153, Co-Mo-W Steel, %
Molybdenum	0.179	8.39
Chromium	0.549	4.14
Nickel	0.563	0.107
Cobalt	...	8.45
Tungsten	...	1.58
Vanadium	0.002	2.04
Manganese	0.867	0.219
Copper	0.089	0.099

The steel samples were dissolved in aqua regia, and repeatedly evaporated with hydrochloric acid to remove nitric acid. In the analysis of the low-molybdenum steel, sample 139, the solution was passed rapidly through Jones reductor to reduce iron(III), and the color was developed in the usual way. From the measured transmittancy, the molybdenum content was determined by reference to the calibration curve. The results are shown in Table V. Analysis of the high-molybdenum steel, sample 153, did not require the removal of iron(III). After dissolving and making up to volume, two aliquots of each sample, of

such volume as would give a final concentration in the optimum range, were developed and measured in the usual way. Table VI gives the results of ten determinations, each involving measurement of two aliquots of the sample solution.

DISCUSSION

The calibration curve for the proposed method is shown in Figure 4, in which per cent absorptancy (100 − % transmittancy) is plotted against log concentration. The maximum attainable photometric accuracy, evaluated from the slope of the curve, is 2.9% relative error per 1% absolute photometric error, conforming closely to derivations from Beer's law (*1*); for an absolute photometric error of 0.2%, the relative analysis error is therefore 0.6%. Maximum accuracy occurs at about 5 p.p.m. of molybdenum, although the accuracy is essentially as good in the range of 2 to 10 p.p.m. The range can be extended to higher concentrations, with an increase in accuracy, by measuring transmittancy ratios (*1*, *5*).

Table V. Analysis of Standard Steel 139

Detn. No.	Sample Wt., G.	Mo Found, %
1	0.2971	0.169
2	0.3229	0.174
3	0.3639	0.208[a]
4	0.2980	0.156
5	0.3398	0.167
6	1.000	0.168
Av.		0.167
Av. deviation		0.005 = 3.0%
Certified analysis by N.B.S.		0.178
Range of results, N.B.S. analysts		0.172–0.180
Av. deviation, N.B.S. analysts		0.002 = 1.1%

[a] Not included in average.

In comparison with the widely used method with tin(II) chloride and thiocyanate, the proposed method has the advantage of greater color stability, and requires no extraction procedure. The sensitivity is comparable to the tin(II) chloride method and to the method of Grimaldi and Wells (*4*), although not quite as high as that of the method of Ellis and Olson (*2*). In the analysis of molybdenum in steels, no interference from iron(III) is encountered unless the ratio of iron to molybdenum exceeds about 20 to 1; the proposed method is therefore especially useful for the analysis of high-molybdenum steels. The usual alloying elements (except vanadium if present in amounts nearly equal to the amount of molybdenum) are without interference. Phosphate, up to at least 1000 p.p.m. as phosphoric acid, did not interfere.

Table VI. Analysis of Standard Steel 153

Detn. No.	Sample Wt., G.	Mo Found, %
1	0.1052	8.37
		8.35
2	0.1904	8.33
		8.33
3	0.1081	8.35
		8.36
4	0.2193	8.22
		8.23
5	0.1231	8.33
		8.33
6	0.1149	8.29
		8.29
7	0.2000	8.36
		8.38
8	0.1051	8.26
		8.26
9	0.1123	8.52
		8.51
10	0.1637	8.38
		8.37
Av.		8.34
Av. deviation		0.05 = 0.65%
Certified analysis by N.B.S.		8.39
Range of results, N.B.S. analysts		8.35–8.45
Av. deviation, N.B.S. analysts		0.4 = 0.48%

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Spectrophotometric Studies on Refined Sugars in Solution

F. W. ZERBAN, LOUIS SATTLER, AND JAMES MARTIN
New York Sugar Trade Laboratory, New York, N. Y.

PETERS and Phelps, of the National Bureau of Standards, were the first to devise a method for the determination of the color of sugars and sugar products, based on strictly scientific principles. The results of these investigations, which were presented at eight meetings of the AMERICAN CHEMICAL SOCIETY between 1921 and 1925, have not been published *in extenso*, but Peters and Phelps (*9*) did present the detailed description of an improved method of preparation and clarification of solutions for color determinations and a discussion of spectrophotometric data, including ratios of absorbancy indexes under various conditions. They concluded from their results that the negative logarithm of the transmittancy for 1-cm. thickness and a concentration of 1 gram of dry substance in 1 ml. of solution ("absorbancy index," a) measured at wave length 560 mμ, is equivalent, colorimetrically, to the integral absorption over the visible spectrum under the same conditions of thickness and concentration, and that therefore the measurement of the absorbancy at that wave length is sufficient for determining the color concentration of the sample without going through the entire spectrum between 400 and 700 mμ. If desired, the various types of coloring matters may be grouped and differentiated by means of the absorption (Q) ratios for selected wave lengths.

In a later publication, Peters and Phelps (*8*) showed that by interpolation between $-\log t$ for the wave lengths 546 mμ and 578 mμ, the $-\log t$ for 560 mμ may be calculated with sufficient precision for all ordinary purposes. Brewster and Phelps (*2*) continued the earlier studies and revised the existing methods of preparing the solutions and certain other details of the manipulation. In addition, Brewster (*1*) described a simplified apparatus for technical use.

Since that time important advances have been made in the determination of the color of many types of materials, and color is now defined as follows (*7*): "Color consists of the characteristics

Peters and Phelps found years ago that the absorbancy index at wave length 560 mμ of sugars and sugar products in solution is equivalent, colorimetrically, to the sum total absorption over the visible range, but the experimental data leading to this conclusion have not been published. As a check, the transmittancy of 60% solutions, filtered through Celite analytical filter aid, of 76 different refined sugars was measured at 20 points from 325 to 825 mμ. The plotted transmittancy curves were smooth but with widely differing slopes. The transmittancy curves of the unfiltered solutions were also plotted, but the results have no physical significance. The brightness, purity, and dominant wave length were computed from the transmittancies of the filtered solutions, and a statistical analysis showed a correlation coefficient of 0.9958 between those three combined and the transmittancy, determined under the same conditions at 560 mμ. Consequently, the corresponding absorbancy index at that wave length gives a measure of the color of the sugar within the limit of error of transmittancy measurements. Absorbancy indexes at any other wave length give erroneous color values. It is concluded that the color of a solution of refined sugar, as perceived by the eye, can be determined by measuring the absorbancy index at a single wave length, 560 mμ, but that determinations at any other wave length give incorrect results as to actual color.

of light other than spatial and temporal inhomogeneities; light being that aspect of radiant energy of which the human observer is aware through the visual sensations which arise from the stimulation of the retina of the eye." The specifications of color as thus defined and approved by the American Standards Association and the National Bureau of Standards read as follows (*7*):

Purpose. To recognize and recommend a basic method for the specification of color, and to facilitate its popular interpretation.

Provisions. (1) The spectrophotometer shall be recognized as the basic instrument in the fundamental characterization of color. (2) Color specifications computed from spectrophotometric data shall be found by means of the standard observer and coordinate system adopted in 1931 by the International Commission on Illumination. In the absence of a special reason for adopting some other illuminant in reducing spectrophotometric data, standard ICI Illuminant C, representative of average daylight, shall be used. The basic specifications of color shall consist of the tristimulus value, Y, and the trichromatic coefficients, or they shall consist of the tristimulus value, Y, and the dominant wave length and purity.

The remaining two paragraphs of the specifications refer to colorimetry by the Munsell system, which is based on the trichromatic system (Y, x, and y).

The above specifications express the color as perceived by the eye of the standard observer under the specified conditions of illumination. Whenever this condition is not fulfilled, the term "color" should not be used, to avoid misunderstanding.

For the reasons indicated, it appeared desirable to check the conclusions of Peters and Phelps by an independent investigation, beginning with refined sugars, and extending it later to other sugar products.

Seventy-six samples of refined sugars were collected, representing all types of such sugars—viz., tablets, coarse, medium, fine, extra fine, and special granulated produced by the refiners in the United States and Canada, as well as confectioners' sugars and off-shore granulated. Solutions of approximately 60° Brix by refractometer were prepared and filtered with specially purified Celite analytical filter aid, as described previously (*10*). Specially prepared asbestos had been recommended by Peters and Phelps (*9*) and by Brewster and Phelps (*2*), but the present authors have shown (*10*) that the use of Celite is justified because asbestos, Celite, and silica gel all act selectively, and the results with asbestos are difficult to reproduce by different workers, because they depend on the porosity of the mat, which varies with the size distribution of the fibers and the pressure applied in the preparation of the mat. These difficulties are largely overcome by the Celite method of the writers. Some form of kieselguhr is now preferred by most of the workers in the field.

The transmittancies of both the unfiltered and the filtered solutions were measured at twenty points between 325 and 825 mμ with the Coleman Universal spectrophotometer in 5-cm. cells, which is the greatest thickness provided for with this instrument. Greater precision would have been obtained with the use of longer cells.

FILTERED SOLUTIONS

The color of these solutions was determined by the method of the American Standards Association (*7*) by determining the trichromatic coefficients according to the selected ordinate method of Hardy (*6*) for Illuminant C, and expressing the results in the monochromatic system. This same method had been used with excellent success by the Java Sugar Experiment Station (*4*) for determining the color of solid refined sugars by reflectance measurements. It was found that ten selected ordinates were sufficient because the transmittancy curves of refined sugars are as shown in Figure 1 for three examples. It was necessary, however, to use transmittancies calculated to 10-cm. thickness at a concentration of 1 gram in 1 ml. of solution, because the brightness (luminance) values on the basis of 1-cm. thickness were too close to 100.

The method of calculating the trichromatic coefficients is illustrated in Table I for sugar 11.

Table I. Calculation of Trichromatic Coefficients by Selected Ordinate Method of Hardy, with Ten Ordinates

Red Primary		Green Primary		Blue Primary	
Selected wave length	T, 10 cm., 1 g./1 ml.	Selected wave length	T, 10 cm., 1 g./1 ml.	Selected wave length	T, 10 cm., 1 g./1 ml.
435.5	70.0	489.4	84.5	422.2	64.5
461.2	78.0	515.1	88.5	432.0	68.7
544.2	91.6	529.8	90.2	438.6	70.8
564.0	93.4	541.4	91.4	444.4	72.8
577.3	94.1	551.7	92.4	450.2	74.8
588.7	94.8	561.9	93.2	455.9	75.1
599.5	95.2	572.5	93.8	462.0	78.3
610.8	95.5	584.8	94.5	468.8	79.7
624.0	96.0	600.7	95.2	477.8	82.0
646.2	96.1	627.1	96.0	495.3	85.4
Total	904.7		919.7		752.1
Factors	0.09804		0.10000		0.11812
Products	88.70		91.97		88.84
Trichromatic coefficients	0.3291		0.3297		0.3412

The transmittancies found for each of the selected wave lengths for each of the three primaries are entered, and added for each primary. The sums are multiplied by the factors shown. Because the green primary of the International Commission on Illumination coincides with the visibility curve for the standard observer, the sum of the transmittancies for the green primary, multiplied by 0.1, gives directly the brightness of the sample. The factors for the red and blue primaries have been computed by Hardy from the tristimulus values and relative energy values for Illuminant C. The trichromatic coefficients are then calculated by expressing the products of the sums and factors as fractions of unity.

To evaluate the stimulus that the eye of the standard observer accepts as equivalent to the trichromatic coefficients under illumination with Illuminant C, the intersection of the trichromatic coefficients of the sample for the red and green primaries is located on the chromaticity diagram (*6*) and a line is drawn from the zero purity point (trichromatic coefficient for the red primary 0.3101, for the green primary 0.3163) through this intersection to the outer contour curve (100 purity). This second intersection indicates directly the dominant wave length. The excitation purity is the percentage ratio of the distance between the zero point and the first intersection to the distance between the zero point and the outer contour curve. The calculation of the brightness has already been described. The writers have not used this graphical method for the determination of dominant wave length and excitation purity from the chromaticity diagram, but have preferred the more exact computational method (*3*).

The brightness, purity, and dominant wave length thus found for each filtered sample are shown in columns 2, 3, and 4 of Table II. The absorbancy indexes, *a*, are given in columns 5, 6, and 7 of the same table for wave lengths 560, 420, and 720 mμ. The samples are arranged in the table in ascending order of *a* at 560 mμ. A comparison between columns 2 and 5 shows that the brightness decreases almost exactly in the same order as the absorbancy index at wave length 560 mμ increases, whereas there is no such relationship between the absorbancy index for 420 mμ, at the blue end, and the brightness. It was therefore de-

Table II. Filtered Solutions in Order of Increasing Absorbancy Index at Wave Length 560 mμ

No.	Brightness	Purity	Dominant Wave Length	*a*, 560 mμ	*a*, 420 mμ	*a*, 720 mμ	*T*, 560, 60 Brix, 5 Cm. Found	*T*, 560, 60 Brix, 5 Cm. Calcd., Eq. 3	Color Gillett	*Q*, 420 mμ / 560 mμ	*Q*, 720 mμ / 560 mμ	*R*, Gillett 560 mμ
45	98.8	4.0	573.9	0.00022	0.00536	0.00000	99.8	100.3	0.00675	24.36	0.00	30.68
14	98.4	5.4	572.8	0.00035	0.00638	0.00000	99.7	100.1	0.00822	23.89	0.00	23.49
39	98.4	5.1	573.3	0.00044	0.00737	0.00000	99.6	100.2	0.01285	16.75	0.00	29.21
51	98.1	4.7	574.1	0.00066	0.00670	0.00000	99.4	100.0	0.01213	10.15	0.00	18.38
70	96.2	8.1	575.0	0.00079	0.00742	0.00000	99.3	99.4	0.01293	9.39	0.00	16.37
28	97.0	8.0	574.4	0.00097	0.01135	0.00000	99.1	99.8	0.01724	11.70	0.00	17.77
24	96.9	7.9	574.3	0.00106	0.01439	0.00000	99.1	99.7	0.01073	13.58	0.00	10.12
17	96.4	5.8	575.2	0.00123	0.00841	0.00009	98.9	99.2	0.00456	6.84	0.07	3.71
29	96.4	6.1	574.4	0.00128	0.00931	0.00000	98.9	99.2	0.01581	7.27	0.00	12.35
15	96.4	6.9	573.4	0.00132	0.01169	0.00000	98.8	99.2	0.01513	8.86	0.00	11.46
71	96.2	8.1	575.3	0.00132	0.01169	0.00000	98.8	99.4	0.01669	8.86	0.00	12.64
43	96.4	4.1	573.0	0.00146	0.00773	0.00000	98.7	98.8	0.01262	5.29	0.00	8.64
56	95.5	5.6	574.4	0.00191	0.00904	0.00017	98.3	98.6	0.01994	4.73	0.09	10.44
2	94.8	9.6	574.1	0.00195	0.01367	0.00052	98.3	98.6	0.01626	7.01	0.27	8.24
18	94.7	10.4	574.1	0.00195	0.01707	0.00000	98.3	98.6	0.01064	8.75	0.00	5.46
6	94.6	7.4	573.7	0.00209	0.01238	0.00110	98.2	98.2	0.01364	5.92	0.53	6.53
41	94.4	7.8	574.6	0.00209	0.01249	0.00031	98.2	98.2	0.01332	5.98	0.15	6.37
16	93.9	9.7	573.6	0.00237	0.01568	0.00031	97.9	98.0	0.02227	6.62	0.13	9.40
72	93.9	10.1	575.0	0.00241	0.01568	0.00000	97.9	98.2	0.02253	6.51	0.00	9.35
52	93.9	9.6	575.4	0.00250	0.01481	0.00000	97.8	98.2	0.01724	5.92	0.00	6.90
21	93.0	14.6	574.2	0.00255	0.02240	0.00022	97.8	98.1	0.01962	8.79	0.09	7.69
73	93.5	11.4	575.3	0.00255	0.01759	0.00000	97.8	98.2	0.02801	6.90	0.00	10.98
60	92.8	12.1	574.8	0.00278	0.01193	0.00000	97.6	97.8	0.02548	4.29	0.00	9.17
47	92.2	17.5	574.2	0.00283	0.02890	0.00000	97.5	98.0	0.03072	10.21	0.00	10.86
67	92.4	11.9	575.4	0.00301	0.01858	0.00000	97.4	97.6	0.02146	6.17	0.00	7.13
11	92.0	11.8	573.9	0.00311	0.01972	0.00150	97.3	97.2	0.02273	6.34	0.48	7.31
44	92.0	12.5	574.5	0.00311	0.01945	0.00000	97.3	97.3	0.02111	6.25	0.00	6.79
55	92.3	10.9	573.7	0.00311	0.01778	0.00000	97.3	97.2	0.01705	5.72	0.00	5.48
46	91.6	13.9	575.1	0.00315	0.02097	0.00000	97.2	97.3	0.02772	6.66	0.00	8.80
37	92.0	9.0	573.3	0.00325	0.01599	0.00164	97.2	96.8	0.01319	4.92	0.50	4.06
50	91.3	16.3	575.1	0.00329	0.02464	0.00000	97.1	97.4	0.02953	7.49	0.00	8.98
12	91.5	11.2	574.9	0.00339	0.01824	0.00159	97.0	96.9	0.02538	5.38	0.47	7.49
34	88.5	17.8	577.3	0.00348	0.02882	0.00048	97.0	96.1	0.02718	8.28	0.14	7.81
40	91.3	11.9	576.7	0.00372	0.01884	0.00000	96.7	97.0	0.02264	5.06	0.00	6.09
32	90.4	14.8	574.1	0.00372	0.02495	0.00000	96.7	96.6	0.02155	6.71	0.00	5.79
7	90.3	13.5	574.2	0.00376	0.02277	0.00141	96.7	96.4	0.03309	6.06	0.38	8.80
31	90.9	12.9	574.4	0.00376	0.02219	0.00013	96.7	96.7	0.02167	5.90	0.03	5.76
68	90.5	14.5	574.7	0.00376	0.02396	0.00079	96.7	96.7	0.03147	6.37	0.21	8.37
69	90.3	12.9	575.4	0.00376	0.02190	0.00000	96.7	96.4	0.02641	5.82	0.00	7.02
36	90.1	15.4	574.1	0.00386	0.02557	0.00057	96.6	96.5	0.02060	6.62	0.15	5.34
38	89.6	17.6	574.1	0.00405	0.03080	0.00013	96.5	96.4	0.02304	7.60	0.03	5.69
35	89.4	19.9	574.6	0.00410	0.03372	0.00000	96.4	96.6	0.03258	8.22	0.00	7.77
23	89.9	12.8	573.6	0.00414	0.02154	0.00017	96.4	96.0	0.02261	5.20	0.04	5.46
57	89.5	18.0	575.1	0.00419	0.02924	0.00000	96.3	96.5	0.03908	6.97	0.00	9.33
49	88.5	22.1	573.6	0.00458	0.04179	0.00000	96.0	96.3	0.03720	9.12	0.00	8.12
19	88.3	18.0	574.8	0.00482	0.02757	0.00026	95.8	95.8	0.02218	5.72	0.05	4.60
27	87.6	19.0	575.6	0.00482	0.03054	0.00075	95.8	95.6	0.02680	6.34	0.16	5.56
22	88.3	13.7	574.7	0.00487	0.02366	0.00083	95.8	95.3	0.01647	4.86	0.17	3.38
3	88.3	12.7	573.1	0.00496	0.02636	0.00057	95.7	95.0	0.03220	5.31	0.11	6.49
13	88.5	12.9	575.1	0.00496	0.02219	0.00057	95.7	95.3	0.02791	4.47	0.11	5.63
25	87.5	17.8	574.8	0.00521	0.02933	0.00101	95.5	95.3	0.03151	5.63	0.19	6.05
8	86.7	20.2	574.3	0.00531	0.03298	0.00191	95.4	95.0	0.04310	6.21	0.36	8.12
33	86.6	21.2	574.7	0.00540	0.03726	0.00066	95.3	95.1	0.03633	6.90	0.12	6.73
61	86.3	24.0	575.3	0.00540	0.03675	0.00022	95.3	95.3	0.02770	6.81	0.04	5.13
62	86.8	18.7	574.8	0.00540	0.03270	0.00114	95.3	95.0	0.03501	6.06	0.21	6.48
84	85.7	22.0	575.0	0.00595	0.04023	0.00000	94.8	94.7	0.03765	6.76	0.00	6.33
81	85.2	21.2	575.5	0.00615	0.03615	0.00137	94.7	94.4	0.03981	5.88	0.22	6.47
53	85.0	21.0	575.1	0.00620	0.03546	0.00119	94.6	94.2	0.04855	5.72	0.19	7.83
83	84.0	22.6	575.5	0.00670	0.03936	0.00173	94.2	93.8	0.04499	5.87	0.26	6.72
74	84.0	23.2	575.5	0.00675	0.04012	0.00052	94.2	93.9	0.04654	5.94	0.08	6.90
48	80.7	28.2	575.9	0.00680	0.04881	0.00110	94.1	92.5	0.05738	7.18	0.16	8.44
63	82.9	23.4	575.0	0.00726	0.04237	0.00141	93.8	93.2	0.04558	5.84	0.19	6.28
58	82.3	24.4	575.2	0.00757	0.04012	0.00223	93.5	93.0	0.04267	5.30	0.29	5.64
82	81.0	19.9	575.2	0.00820	0.03536	0.00101	93.0	91.7	0.03679	4.31	0.12	4.49
1	80.4	25.5	576.3	0.00846	0.04353	0.00255	92.8	92.1	0.05399	5.15	0.30	6.38
59	85.2	24.9	575.4	0.00851	0.04045	0.00101	92.7	94.8	0.08391	4.75	0.12	9.86
66	80.7	28.4	572.0	0.00851	0.06819	0.00101	92.7	92.2	0.06987	8.01	0.12	8.21
20	80.6	29.9	575.6	0.00851	0.05452	0.00061	92.7	92.6	0.03808	6.41	0.07	4.58
26	78.6	29.8	575.3	0.00931	0.05331	0.00092	92.1	91.4	0.04161	5.73	0.10	4.36
54	78.5	27.6	575.7	0.00947	0.04802	0.00022	91.9	91.1	0.05516	5.07	0.02	5.83
9	68.7	53.8	575.7	0.01415	0.09872	0.00292	88.2	88.3	0.12182	6.98	0.21	8.61
64	50.1	48.8	575.9	0.02865	0.11739	0.01062	77.5	76.7	0.08205	4.10	0.37	2.86
42	48.6	63.1	578.2	0.02959	0.10132	0.00783	76.9	77.7	0.09843	3.42	0.26	3.33
65	49.0	54.2	576.7	0.02976	0.13872	0.00809	76.8	76.8	0.09235	4.66	0.27	3.10
30	43.5	58.1	578.2	0.03478	0.13188	0.00958	73.4	74.1	0.06521	3.79	0.28	1.88
85	35.5	61.3	576.2	0.04342	0.16576	0.01972	68.0	69.5	0.07515	3.82	0.45	1.73

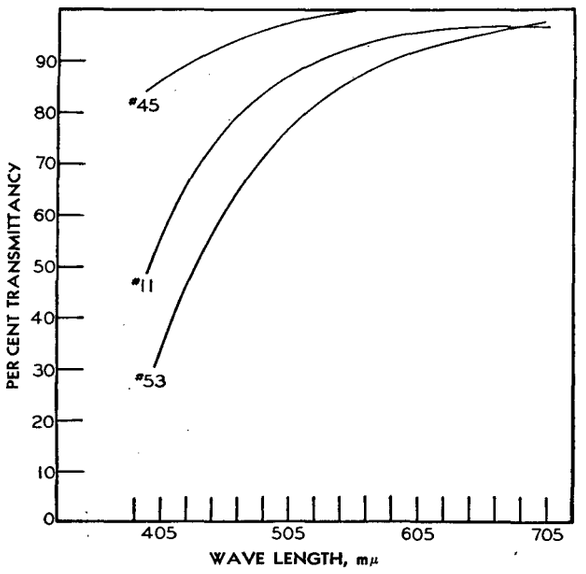

Figure 1. Transmittancy Curves of Three Refined Sugars

cided to make a statistical analysis of the relation between the absorbancy index at 560 mμ, and the color analysis as presented by brightness, purity, and dominant wave length. Irving Lorge, of Teachers College, Columbia University, kindly offered to have this statistical analysis made with modern machines. The equation derived from the experimental data is as follows, for the filtered solutions:

$$a,\ 560\ m\mu = 0.169128 - 0.00084\ \text{brightness} - 0.00021\ \text{purity} - 0.00015\ \text{dominant wave length} \quad (1)$$

The correlation coefficient between the absorbancy index at 560 mμ and the monochromatic analysis is 0.9927.

Because the dominant wave length (column 4) varies only a few units around 575 mμ, it was felt that this criterion might be omitted. Under this condition the following formula was obtained for the filtered solutions:

$$a,\ 560\ m\mu = 0.083091 - 0.00084\ \text{brightness} - 0.00022\ \text{purity} \quad (2)$$

The correlation coefficient in this case is 0.9925, only a shade lower than when the entire monochromatic analysis is taken into account.

Considering that the absorbancy indexes are logarithms, a better correlation should be expected between the transmittancies observed at 560 mμ and the monochromatic analysis. The calculations made by Lorge bore out this expectation. The transmittancies found for the 5-cm. cell had to be corrected to exactly 60.00° refractometric Brix (0.7719 gram of dry substance in 1 ml.) because the filtered solutions had a higher Brix, 61° to 62°, due to evaporation during the filtration. The corrected observed transmittancies at 560 mμ are shown in column 8 of Table II.

These values were compared statistically with the results of the monochromatic analyses, yielding the following equation:

$$T\ 560,\ 5\ \text{cm.},\ 60\ \text{Brix} = 0.5938\ \text{brightness} + 0.1149\ \text{purity} + 0.0901\ \text{dominant wave length} - 10.5107 \quad (3)$$

with a correlation coefficient of 0.9958, which, from a statistical viewpoint, is much better than that for Equation 1. The transmittancies calculated from the monochromatic analysis, according to this equation, are shown in column 9 of Table II. The differences between the found and calculated values average 0.39% transmittancy, and exceed 1% in only three cases. These differences are, on the whole, well within the limit of error of transmittancy measurements in the high range, mostly above 90%.

If the transmittancy at 560 mμ, 5 cm., 60° Brix, is correlated only with brightness and purity, leaving out the dominant wave length, the equation becomes:

$$T\ 560,\ 5\ \text{cm.},\ 60\ \text{Brix} = 0.5925\ \text{brightness} + 0.1184\ \text{purity} + 41.3235 \quad (4)$$

in which case the correlation coefficient is 0.9957, again nearly the same as when all three variables of the monochromatic analysis are used. If the purity is omitted as criterion, and only brightness and dominant wave length are considered, the correlation coefficient drops to 0.9936, and for the brightness alone to 0.9933.

The conclusion to be drawn from the data is that the absorbancy index of the filtered solution, determined at wave length 560 mμ, is equivalent to the color of a white sugar in solution, as originally found by Peters and Phelps. Measurements at any other individual wave length give only absorption values, not color values. Peters and Phelps have explained this fact by the observation that the absorption curves, reduced to unit color concentration, of all the white sugars studied by them cross at wave length about 560 mμ. Toward the blue end of the spectrum all the absorbancy indexes rise to different heights, depending on the samples, while toward the red end of the spectrum they fall, also to different values. These rises and falls are expressed numerically by the ratios of the absorbancy index at any wave length other than 560 mμ to that at 560 mμ. Wave length 420 mμ, at the extreme blue end of the visible spectrum, and wave length 720 mμ, just beyond its red end, were chosen to calculate these Q-ratios, which are shown in columns 11 and 12 of Table II. It is seen that the Q-ratios for 420/560 vary from 3.42 to as high as 24.36, and those for 720/560 from 0.00 to 0.50. This explains why the absorbancy indexes for 420 mμ do not vary in the same order as those for 560 mμ. They are not a measure of color, but merely of absorption at 420 mμ. The same is true of any other wave length except 560 mμ.

A further study of the Q-ratios shows that, as those for 420/560 rise, those for 720/560 fall in a strikingly regular way, as is seen in Table III.

Table III. Comparison of Q-Ratios 420/560 with Q-Ratios 720/560

Q-Ratios 420/560	Average Q-Ratios 720/560
3.00–3.99	0.33
4.00–4.99	0.19
5.00–5.99	0.15
6.00–6.99	0.12
7.00–7.99	0.09
8.00–8.99	0.05
9.00 and above	0.00

Column 10 of Table II, marked Color Gillett, gives the results of the method of Gillett, Meads, and Holven (*5*) for determining the color of white sugars by measuring the absorbancy index of the unfiltered solution at 420 and 720 mμ, and deducting twice the latter value from once the former value. The figures thus calculated have been placed in Table II to permit direct comparison with the color values found by the measurements upon the filtered solutions. This color Gillett places the samples in an order different from that based on the monochromatic analysis of the entire transmittancy curve, or that based on the absorbancy index at 560 mμ. This was to be expected, because as is shown by the results obtained with the unfiltered solutions (Table V), the absorbancy index at 420 mμ is very high as compared with that at 720 mμ, so that the final color value of Gillett will be roughly comparable to the absorbancy index at 420 mμ of the filtered solution, but very much higher, and in an entirely different order of sequence, than the color as expressed either by the

absorbancy index at 560 mμ or by the monochromatic analysis of the transmittancy curve. This is well illustrated in column 13 of Table II, which shows the ratios of the color Gillett to the absorbancy indexes for 560 mμ of the filtered solution. These ratios in condensed form are compared in Table IV with the Q-ratios 420/560 for the filtered solutions.

The two ratios increase in a similar fashion, showing that the color Gillett varies roughly in the same order as the absorbancy index of the filtered solution at 420 mμ.

It appears from the figures in Table IV that, if the ratios of the color Gillett to the absorbancy index at wave length 560 mμ of the filtered solution of different samples are equal among themselves, the Q-ratios 420/560 should also be approximately equal, and in that case the color Gillett would be proportional to the absorbancy index of the filtered solution at 560 mμ. Such a condition may be expected when refined sugars are produced by the same refining process from raws of similar types, and in this case the simple and rapid method of Gillett, Meads, and Holven is of distinct practical value, but the result should not be called color. The writers (*11*) fell into the same error when they devised a method for computing the "color" and turbidity of raw and refined sugars from the transmittancy and Tyndall beam intensity of unfiltered solutions, measured at 529 mμ with the Pulfrich photometer because the absolute turbidity of the standard block was known only at that wave length. It is now intended to repeat the earlier work on a spectrophotometric basis.

Table IV. Comparison of Q-Ratios for Filtered Solutions

Q-Ratio 420/560	Av. Ratio of Color Gillett to Absorbancy Index at 560 mμ[a]
3.00-3.99	2.31
4.00-4.99	5.81
5.00-5.99	6.26
6.00-6.99	7.27
7.00-7.99	8.74
8.00-8.99	8.72
9.00 and above	19.22

[a] From column 13, Table II.

Table V. Unfiltered Solutions in Order of Increasing Absorbancy Index at Wave Length 560 mμ

No.	Brightness	Purity	Dominant Wave Length	a, 560 mμ	a, 420 mμ	a, 720 mμ	T, 560, 60 Brix, 5 Cm. Found	T, 560, 60 Brix, 5 Cm. Calcd., Eq. 7	Q, 420 mμ / 560 mμ	Q, 720 mμ / 560 mμ
18	90.3	7.5	574.8	0.00410	0.01777	0.00218	96.4	99.7	4.33	0.53
45	88.8	5.2	575.6	0.00506	0.01175	0.00250	95.6	97.2	2.32	0.49
52	87.1	11.9	576.0	0.00560	0.02034	0.00155	95.1	96.6	3.63	0.28
43	84.9	8.6	576.1	0.00670	0.01986	0.00362	94.2	94.4	2.96	0.54
21	84.3	15.1	575.0	0.00685	0.02612	0.00325	94.1	96.8	3.81	0.47
69	84.4	16.1	576.0	0.00685	0.02959	0.00159	94.1	95.5	4.32	0.23
19	83.9	16.8	575.0	0.00691	0.02924	0.00353	94.0	96.8	4.23	0.51
24	84.3	10.7	575.4	0.00696	0.02007	0.00467	94.0	95.4	2.88	0.67
39	84.6	9.4	576.9	0.00696	0.01785	0.00250	94.0	93.2	2.56	0.36
15	84.0	11.6	575.0	0.00716	0.02219	0.00353	93.8	96.0	3.10	0.49
2	83.3	13.0	575.1	0.00742	0.02426	0.00400	93.6	95.6	3.27	0.54
29	83.4	11.4	576.1	0.00742	0.02147	0.00283	93.6	93.9	2.89	0.38
6	83.2	11.4	574.8	0.00762	0.02270	0.00453	93.5	95.7	2.98	0.60
51	83.1	10.0	576.1	0.00768	0.01965	0.00376	93.4	93.5	2.56	0.49
28	82.7	11.1	575.7	0.00773	0.02262	0.00269	93.4	94.0	2.93	0.35
16	82.3	16.0	575.7	0.00783	0.02857	0.00315	93.3	94.5	3.65	0.40
32	82.1	15.9	574.4	0.00783	0.02907	0.00376	93.3	96.3	3.71	0.48
55	82.4	12.9	575.1	0.00794	0.02495	0.00395	93.2	95.0	3.14	0.50
71	82.3	11.9	575.3	0.00804	0.02299	0.00315	93.1	94.4	2.86	0.39
44	81.7	14.9	575.7	0.00825	0.02741	0.00315	92.9	94.0	3.32	0.38
41	81.4	11.6	575.5	0.00846	0.02306	0.00487	92.8	93.5	2.73	0.57
72	79.7	15.4	575.7	0.00921	0.02959	0.00353	92.1	92.7	3.21	0.38
31	80.1	14.2	576.0	0.00926	0.02807	0.00320	92.1	92.3	3.03	0.35
17	80.0	7.6	574.9	0.00931	0.01898	0.00721	92.1	92.8	2.04	0.77
40	79.3	15.6	576.4	0.00953	0.02950	0.00343	91.9	91.5	3.10	0.36
62	79.0	21.7	575.4	0.00953	0.04123	0.00311	91.9	93.8	4.33	0.33
56	78.5	14.5	575.5	0.01007	0.02978	0.00492	91.4	92.1	2.96	0.49
34	76.0	18.8	577.5	0.01101	0.03556	0.00419	90.7	88.2	3.23	0.38
73	76.4	18.6	576.1	0.01107	0.03487	0.00343	90.6	90.5	3.15	0.31
22	76.3	13.8	575.4	0.01118	0.02967	0.00660	90.5	90.6	2.65	0.59
33	75.7	22.5	576.6	0.01141	0.04283	0.00325	90.4	89.9	3.75	0.28
70	76.0	10.7	576.5	0.01152	0.02807	0.00757	90.3	88.3	2.44	0.66
47	74.9	21.0	575.7	0.01181	0.04134	0.00531	90.0	90.5	3.50	0.45
35	74.8	21.6	576.0	0.01186	0.04202	0.00472	90.0	90.1	3.54	0.40
38	74.7	17.3	574.8	0.01203	0.03696	0.00696	89.9	91.1	3.07	0.58
63	73.3	26.2	575.8	0.01267	0.05406	0.00424	89.4	90.2	4.27	0.33
37	73.8	13.1	576.3	0.01278	0.02865	0.00773	89.3	87.5	2.24	0.60
11	73.0	19.4	575.8	0.01290	0.03809	0.00768	89.2	88.8	2.95	0.60
14	72.9	11.4	574.1	0.01319	0.02782	0.00980	88.9	89.9	2.11	0.74
50	72.6	21.0	576.0	0.01325	0.04123	0.00585	88.9	88.5	3.11	0.44
12	72.3	18.3	575.1	0.01361	0.03788	0.00625	88.6	89.2	2.78	0.46
46	71.3	19.6	576.8	0.01397	0.04012	0.00620	88.3	86.2	2.87	0.44
60	71.3	17.3	574.5	0.01403	0.03990	0.00721	88.3	89.3	2.84	0.51
81	70.9	26.7	576.1	0.01409	0.05171	0.00595	88.2	88.2	3.67	0.42
57	70.9	23.9	576.6	0.01421	0.04776	0.00434	88.1	87.0	3.36	0.31
68	68.5	21.6	576.4	0.01580	0.04559	0.00706	86.9	85.3	2.89	0.45
7	67.4	24.3	576.4	0.01630	0.04949	0.00820	86.5	85.0	3.04	0.50
3	67.6	20.4	575.0	0.01637	0.04724	0.00752	86.5	86.6	2.82	0.46
1	66.2	33.2	575.6	0.01675	0.06861	0.00731	86.2	86.9	4.10	0.44
58	66.1	29.1	576.4	0.01675	0.05719	0.00726	86.2	85.0	3.41	0.27
20	66.3	26.0	576.5	0.01694	0.05670	0.00931	86.0	84.7	3.35	0.55
27	66.1	22.2	575.7	0.01720	0.04510	0.00915	85.8	84.9	2.62	0.53
23	65.8	18.7	576.0	0.01752	0.04145	0.00942	85.6	83.6	2.37	0.54
61	65.3	26.5	576.8	0.01772	0.04306	0.00768	85.4	83.4	2.43	0.43
13	63.5	23.2	576.1	0.01858	0.04949	0.01079	84.8	82.7	2.66	0.58
82	63.8	25.6	576.2	0.01864	0.05287	0.00804	84.7	83.2	2.84	0.43
49	63.5	26.3	576.2	0.01904	0.05670	0.00975	84.4	83.1	2.98	0.51
36	61.9	20.6	576.1	0.02007	0.04698	0.01319	83.7	81.2	2.34	0.66
67	61.0	20.3	576.6	0.02090	0.04750	0.01302	83.0	79.8	2.27	0.62
25	60.4	26.5	572.4	0.02097	0.05331	0.01090	83.0	79.3	2.54	0.52
83	58.7	31.6	576.7	0.02211	0.06635	0.01068	82.2	80.1	3.00	0.48
8	57.7	33.3	576.4	0.02240	0.06716	0.01203	82.0	80.1	3.00	0.54
84	56.5	28.6	576.4	0.02366	0.06345	0.01290	81.0	78.5	2.68	0.55
53	55.7	34.9	576.0	0.02419	0.07423	0.01284	80.7	79.7	3.07	0.53
54	55.4	33.6	576.0	0.02449	0.07520	0.01002	80.4	79.2	3.07	0.42
26	53.4	32.1	578.5	0.02612	0.06799	0.01319	79.3	74.0	2.60	0.50
74	52.8	33.9	577.4	0.02700	0.07352	0.01349	78.7	75.5	2.72	0.50
66	48.6	36.2	574.7	0.03054	0.10223	0.01618	76.2	77.1	3.35	0.53
48	48.8	41.0	576.7	0.03063	0.08762	0.01512	76.2	75.1	2.86	0.49
59	43.0	53.7	578.5	0.03536	0.11427	0.01518	73.0	70.7	3.23	0.43
9	32.5	69.2	579.5	0.04622	0.16576	0.02197	66.3	64.9	3.59	0.48
42	22.6	64.8	579.7	0.06308	0.16383	0.03270	57.1	57.3	2.60	0.52
64	20.3	56.0	577.9	0.06799	0.16383	0.04089	54.6	57.0	2.41	0.60
30	10.0	58.8	584.1	0.10000	0.17959	0.05719	41.1	41.4	2.54	0.57
65	9.1	69.3	583.1	0.10410	0.20969	0.05867	39.6	44.1	2.01	0.56
85	6.5	58.5	581.6	0.11871	0.22219	0.07352	34.0	42.8	1.87	0.62

UNFILTERED SOLUTIONS

In order to obtain the necessary values of the color Gillett, transmittancy measurements were made upon the unfiltered solutions throughout the spectral range, and the color was expressed in the monochromatic system of brightness, purity, and dominant wave length. These values have, however, no specific physical significance because they represent the sum total effect of the absorption by the coloring matter plus turbidity, and the scattering and reflectance effects of the particles causing turbidity. The results are shown in Table V, which is arranged in the same manner as Table II, except that columns 10 and 13 are omitted.

Despite the disturbing effects caused by the turbidity, the absorbancy index at 560 mμ increases in roughly the same order as the brightness decreases. Lorge has established, in the same manner as for the filtered solutions,

equations for the relation between the absorbancy index at 560 mμ on the one hand, and the brightness, purity, and dominant wave length on the other. The equation corresponding to Equation 1 is:

$$a, 560\ m\mu = -0.00114 \text{ brightness} - 0.00047 \text{ purity} + 0.00380 \text{ dominant wave length} - 2.08193 \quad (5)$$

If the dominant wave length is again omitted, the equation is:

$$a, 560\ m\mu = -0.00140 \text{ brightness} - 0.00043 \text{ purity} + 0.12477 \quad (6)$$

Table VI. Comparison of Q-Ratios for Unfiltered Solutions

Q-Ratios 420/560	Av. Q-Ratios 720/560
1.87-2.49	0.61
2.50-2.99	0.50
3.00-3.49	0.44
3.50-3.99	0.41
4.00-4.99	0.40

The correlation coefficient for Equation 5 is 0.9622, and for Equation 6 it is 0.9468; both figures, as expected, are considerably lower than for the corresponding Equations 1 and 2 for filtered solutions.

For reasons explained previously, equations were established correlating the monochromatic analysis with the transmittancies for 560 mμ, 5 cm., 60 Brix, rather than with the corresponding absorbancy indexes. This gave the following formulas:

$$T\ 560, 5 \text{ cm.}, 50 \text{ Brix} = 0.6622 \text{ brightness} + 0.1683 \text{ purity} - 1.4832 \text{ dominant wave length} + 891.2300 \quad (7)$$

$$T\ 560, 5 \text{ cm.}, 60 \text{ Brix} = 0.7619 \text{ brightness} + 0.1509 \text{ purity} + 30.0402 \quad (8)$$

The correlation coefficient for Equation 7 is 0.9860, much higher than for Equation 5, but much lower than for Equation 3. Similarly, the correlation coefficient for Equation 8 is 0.9792, much higher than for Equation 6, but much lower than for Equation 4. If the purity is omitted as a criterion, and only brightness and dominant wave length are considered, the correlation coefficient is 0.9839, and for the brightness alone it drops to 0.9776.

The transmittancies at 560 mμ for the 5-cm. cell and 60 Brix are shown in column 8, and those calculated from Equation 7 in column 9 of Table V. As expected, the discrepancies are much greater than for the filtered solutions, averaging 1.55% transmittancy, with a maximum of 8.8%, proving that turbid solutions are not suitable for color determinations because the transmittancy is affected by the scattering and reflectance effects of the turbidity particles.

The absorbancy indexes for wave length 420 mμ (column 6) are again in an order entirely different from that of the absorbancy indexes at 560 mμ (column 5). This is well shown by the Q-ratios for 420/560 (column 10), and also in Table VI, which gives a comparison, in condensed form, with the Q-ratios 720/560 (column 11).

The range of the Q-ratios 420/560 is numerically very much lower than for the filtered solutions (Table III), and conversely the range of those for 720/560 is considerably higher, because of the effect of the turbidity. The Q-ratios for 720/560 again change in the reverse order as those for 420/560, but the lowest average ratio for 720/560 (0.40) is considerably higher than the highest average Q-ratio for the filtered solutions (0.33).

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Spectrophotometric Analysis of Accelerator-Rubber Mixtures

K. E. KRESS, *Firestone Tire and Rubber Co., Akron, Ohio*

RECENT trends have emphasized use of physical or instrumental methods as auxiliary or alternative methods for established chemical procedures. The ultraviolet spectrophotometer offers a sensitive, accurate, and particularly rapid method of analysis well adapted to control of many organic rubber compounding ingredients, especially where available chemical methods may be inadequate, or complex and time-consuming.

The application of ultraviolet spectroscopy to analysis of synthetic elastomers (*25*) and determination of their antioxidant content (*4*) has been described. However, relatively little has been done with the organic accelerators, those important compounding ingredients essential for obtaining rapid vulcanization and optimum strength in most rubber stocks.

Some early work with ultraviolet spectroscopy in determining the degree of accelerator combination with rubber on vulcanization, and the effect of cure on the structure of accelerators, has been reported (*10*). An investigation of the transformation which tetramethylthiuram disulfide undergoes during the vulcanization process has been carried out following the spectrographic technique (*19*). More recently (*20*) the ultraviolet spectral absorption curves of several commercially important rubber accelerators have been interpreted in the light of their known chemical structure, or the spectral curves were considered as a guide in confirming the molecular structure of the accelerators. Though some quantitative absorption data were reported, no rigorous investigation of the quantitative aspects of accelerator absorption has been undertaken, the emphasis heretofore being on the qualitative data of the spectral curve. Most of the published quantitative work concerned purified accelerators, and no consideration was made of mixed accelerators or of such compounds when combined with rubber.

For this investigation it was deemed advisable to select a few

Table I. Specific Extinction Coefficients at Wave Lengths of Maximum Absorption for Various Accelerators

Trade Name	Chemical Name		252 mμ	258 mμ	274 mμ	280 mμ	282 mμ	329 mμ
Captax	Mercaptobenzothiazole		45.4	33.8	9.6	11.2	12.2	154.0
MBT	(2-thiazolethiol)		47.9	35.6	11.6	....	13.6	154.1
Thiotax		Av.	46.7		10.6			154.0[a]
Altax	Benzothiazyldisulfide		41.0	45.2	61.1	58.2	55.8	10.6
MBTS			42.7	47.0	63.0	59.8	57.5	11.3
Thiofide		Av.		46.1	62.1[a]	59.0		
2-MT	2-Mercaptothiazoline		48.5	49.1	106.0	123.0	120.8	0.12
	(thiazoline-2-thiol)		49.7	49.3	104.8	120.8	119.0	....
		Av.				121.9[a]		
Methyl Tuads	Tetramethylthiuram disulfide		52.0	49.5	47.0	47.6	47.7	2.5
Thiuram M	[bis (dimethylthiocarbamyl)		53.1	50.9	48.0	48.8	48.7	2.6
Thiurad	disulfide]	Av.					48.2[a]	
Tuex								
Monex	Tetramethylthiuram monosul-		37.9	47.1	73.2	77.6	78.2	10.1
Thionex	fide		37.9	47.1	73.5	78.7	78.9	9.6
	[bis (dimethylthiocar- bamyl)sulfide]	Av.				78.2	78.6[a]	
DPG	Diphenylguanidine		65.4	69.5	55.5	45.9	42.4	0.18
			66.8	69.5	52.6	43.1	39.9	0.52
		Av.		69.5[a]				0.35
DOTG	Di-*o*-tolylguanidine		51.4	49.0	28.2	21.6	19.7	0.23
			50.4	46.8	26.8	20.5	18.6	0.11
		Av.	50.9[a]		27.5			0.17

[a] Wave length of maximum absorption for accelerator listed. Above data obtained with two different weighings on two different dates, using same sample of accelerator of commercial purity.

rubber accelerators representative of the main commercial classes in use in the rubber industry today. A list of seven such accelerators from the thiazole, thiuram, guanidine, and thiazoline classes is given in Table I. These accelerators were the regular commercial grades (*18*). To check their purity and identity, the melting points were obtained for comparison with published data. An additional proof of identity was the color reaction with cobalt oleate (*28*) used as detailed by the "Vanderbilt Rubber Handbook" (*27*). Observed colors with cobalt oleate were the same as those reported for the particular type of accelerators involved. A strong light blue color reaction of 2-mercaptothiazoline was noted, which has not been previously reported.

The instrument used in this investigation was the Beckman Model DU ultraviolet spectrophotometer, whose construction and optimum conditions of operation have been described in detail (*5, 8, 16, 17*). An experimental calibration of the spectrophotometer photometric circuit was carried out just prior to this investigation following an established procedure (*31*). Results with a potassium acid phthalate standard proved the instrument in use to be functioning in a manner comparable to the reported average of a total of 24 other Beckman spectrophotometers (*12, 24*).

In operation for quantitative analysis, the sensitivity control was set near the three turns counterclockwise position within the recommended range of optimum accuracy and the instrument was "zeroed" with the slit width mechanism. This gave the minimum slit for a setting yielding optimum accuracy and was considered the best balance of slit width and sensitivity setting for quantitative analysis of accelerator solutions.

SOLVENT SELECTION

Commonly useful solvents for the ultraviolet spectral region have been given in several publications (*1, 6, 22, 23*) and desirable solvent properties have been listed. The following solvents were investigated: the paraffins *n*-heptane, "iso-octane" (2-methylheptane), cyclohexane, and methylcyclohexane; ethyl and methyl alcohols; diethyl and dioxane ethers; benzene and chloroform. From this list of solvents, C.P. reagent grade chloroform was selected as the most promising, primarily because it showed excellent solubility characteristics for all accelerators and elastomers at room temperature. Its nonflammable character and availability at moderate cost, in a grade suitable for spectral analysis without further purification, also favored its selection. The main disadvantages of chloroform are relatively high volatility and toxicity. When solution is completed on short standing in stoppered flasks and spectral data are recorded rapidly, the high volatility need cause no concern. Most organic spectral solvents are toxic to some extent, and in any case ventilation must be provided to reduce this factor to a safe level.

The lower limit of transparency of C.P. chloroform is near 244 mμ, which was found satisfactory for all accelerators examined, because their ultraviolet absorption maxima were all located well above this limit in chloroform.

The effect of different solvents on selectivity (number and type of maxima) and intensity of spectral absorption is an important factor in selection of a solvent for spectral analysis. Presence of a strongly absorbing and fairly sharp maximum, preferably in the longer wave length region of the ultraviolet (where narrow slit widths can be used with optimum sensitivity, and interfering absorption is usually lower) are points to be sought.

Because of good transparency to ultraviolet light, coupled with high solubility for the alkaline pigment, the spectral curves of di-*o*-tolylguanidine in chloroform, ethyl ether, and aqueous 1% hydrochloric acid were obtained and are illustrated in Figure 1. Differences are noted in both selectivity or curve shape and in-

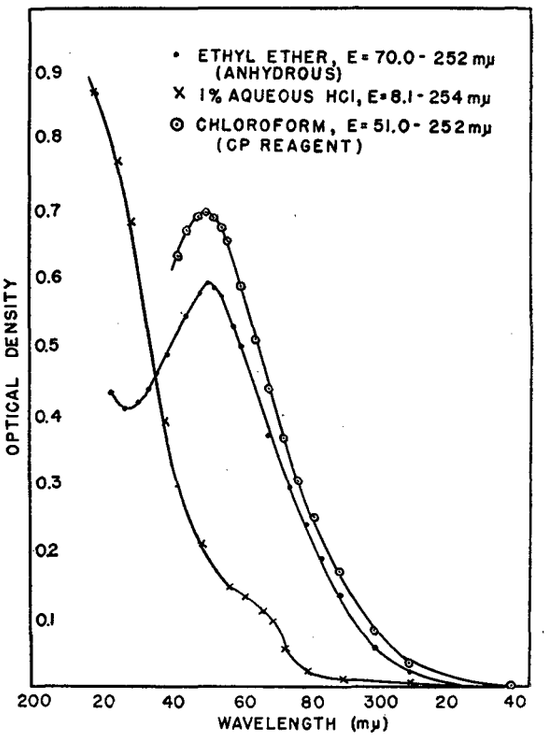

Figure 1. Effect of Solvent on Di-*o*-tolylguanidine Spectral Absorption

Ultraviolet spectrophotometric analysis of mixtures of seven common organic accelerators in rubber is described. Chloroform solutions of 10 to 25% accelerator as a master batch stock containing a single- or a two-component accelerator were analyzed with a degree of rapidity, accuracy, and precision believed adequate for routine control. Emphasis is placed on ease and adaptability of the method for analysis of two-component accelerator stocks. Absolute and relative compositions were determined by accepted mathematical procedures.

tensity of absorption, as measured by the specific extinction coefficient, E. Though the selectivity of ether and chloroform solutions is similar, with maximum at 252 to 254 mμ, the intensity of absorption of di-*o*-tolylguanidine in ethyl ether (E-70.0) is 37% greater than in chloroform (E-51.0). In the case of 1% hydrochloric acid, the curve shape of di-*o*-tolylguanidine has been greatly altered and the E value lowered. The acid solution represents a reaction with the alkaline di-*o*-tolylguanidine, forming di-*o*-tolylguanidine hydrochloride, while chloroform and ethyl ether form more or less normal solutions.

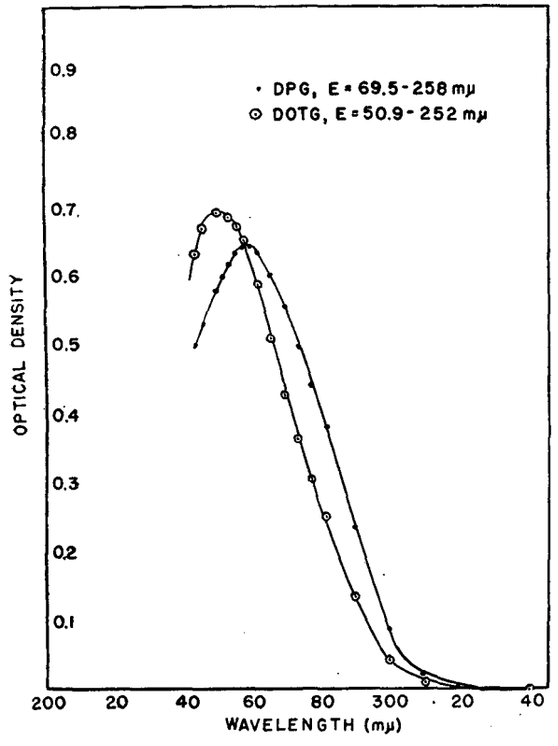

Figure 2. Guanidine-Type Accelerators in Chloroform

Solutions of mercaptobenzothiazole had essentially the same E value in methanol (E-152) as in chloroform (E-154), but character and wave length of the absorption maximum shifted from a sharp 329 mμ maximum in chloroform to a broader 323 mμ maximum in methanol, though general spectral curve shape is otherwise similar. Differences such as this wave-length shift have been attributed to the greater polarity of the alcohol (*6*).

These data emphasize the need for careful solvent selection, because different solvents may affect spectral absorption considerably, and change of solvent will make necessary a recalibration.

SPECTRAL ABSORPTION OF MASTER BATCH COMPONENTS

Selectivity. The ultraviolet absorption curves of chloroform solutions of the seven accelerators described in Table I are illustrated in Figures 2, 3, 4, and 5. The guanidine accelerators, DPG and DOTG of Figure 2, have their absorption maxima near the lower limit of ultraviolet transparency of chloroform (244 mμ). The wave length of absorption maxima of diphenylguanidine (258 mμ) and di-*o*-tolylguanidine (252 mμ) differ by only 6 mμ, and their general character is similar.

The selective absorption of the thiazole accelerators, mercaptobenzothiazole and benzothiazyl disulfide, are illustrated in Figure 3. Two molecules of mercaptobenzothiazole are joined by a disulfide linkage to form benzothiazyl disulfide. Their selective absorption is different, however, the benzothiazyl disulfide maximum being at 274 mμ, while the unique sharp maximum of mercaptobenzothiazole is positioned at 329 mμ. It is of interest to note that mercaptobenzothiazole minimum absorption at 272 to 274 mμ is in the range of the benzothiazyl disulfide maximum.

Though the only difference between tetramethylthiuram monosulfide and tetramethylthiuram disulfide is one more sulfur atom in the disulfide linkage of the latter, the spectral curves of Figure 4 are distinctly different.

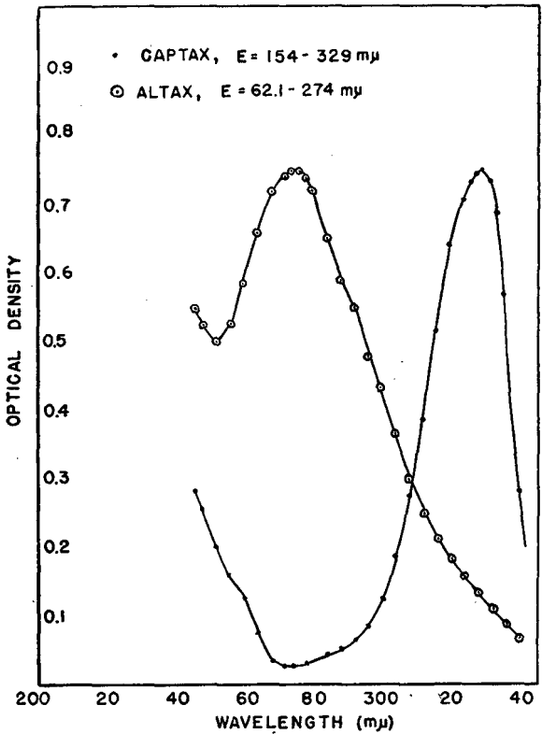

Figure 3. Thiazole-Type Accelerators in Chloroform

The absorption curve of 2-mercaptothiazoline, as illustrated in Figure 5, resembles that of tetramethylthiuram monosulfide, though the maximum of 2-mercaptothiazoline (280 mμ) is sharper.

Intensity. The intensity of absorption of the seven accelerator pigments in chloroform is listed in Table I. An accepted measure

for quantitative analysis of materials is the specific extinction coefficient (*6, 15, 22, 29*), designated here as E value. This factor has been determined at the wave length of the most prominent absorption of the accelerators. It is seen that intensity of absorption at the maxima ranges from a high of 154.0 for mercaptobenzothiazole at 329 mμ to a low of 48.2 for tetramethylthiuram disulfide at 282 mμ. Thus, all seven accelerators absorb relatively strongly at their maxima, which is conducive to determination in a single-accelerator master batch with good accuracy.

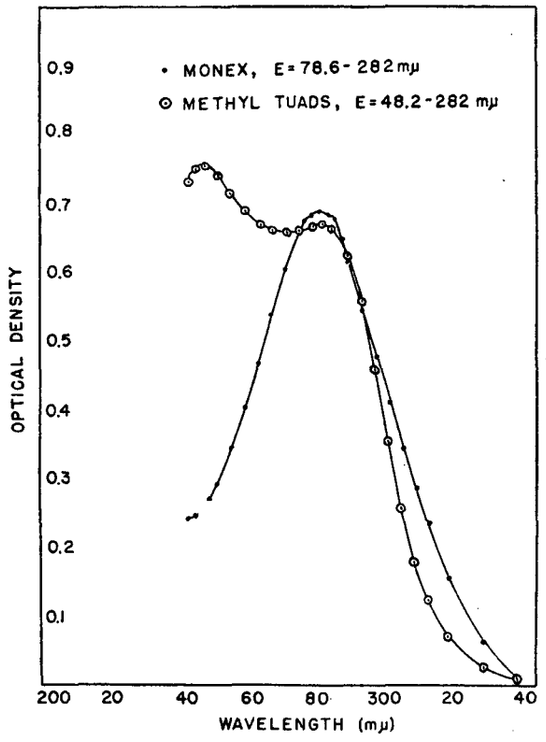

Figure 4. Thiuram Sulfide-Type Accelerators in Chloroform

One factor which may affect accuracy, that is not considered in the data of Table I but would be present in routine control of accelerator master batches, is the variation in spectral purity of the accelerators going into the master batch. Some variation in spectral characteristics may be expected from products of different suppliers, and for the same product if unstable in storage. These factors are not fully evaluated in this report and need further investigation. However, the particular pigments used here are considered near the average of the commercial accelerators available, and it is believed that if the acceptable commercial accelerator purity limits are controlled spectrophotometrically, the analysis of master batches will not be adversely affected by normal differences in accelerator purity any more than when chemical methods of control are used.

Light Stability. Several factors that may affect the stability of solutions have been enumerated (*1*). One of the purposes of examining solutions prepared at room temperature was to eliminate the heating or temperature effect. Light stability was checked by exposing chloroform solutions of the accelerators, diluted to a strength suitable for direct spectral comparison, in clear glass-stoppered flasks to normal changes of daylight (no direct sunlight) and darkness for 50 hours. The shape and intensity of the spectral curves were affected in only two cases. The spectral curves indicate that tetramethylthiuram monosulfide may be partially oxidized to tetramethylthiuram disulfide or decomposes, while the benzothiazyl disulfide clearly was partially reduced to mercaptobenzothiazole, resulting in a 158% increase in absorption at 329 mμ. One obvious solution for such fading due to light is to store the solutions in a dark place, such as a cabinet. A test of this procedure proved that with as much as 32 hours' standing in the dark, benzothiazyl disulfide and tetramethylthiuram monosulfide underwent no appreciable change in curve shape, and drop in intensity of absorption was of the same order (1 to 3%) or less than that for other accelerators whose solutions were found to be relatively stable to light.

It is known that pure chloroform is liable to spontaneous decomposition, especially when exposed to light and air. Such decomposition is prevented to a great extent by the presence of a small amount of alcohol, and accordingly, chloroform contains from 0.5 to 1.0% alcohol (*2, 9*). The reagent grade chloroform used in this investigation contained approximately 0.75% ethyl alcohol.

Absorption of Elastomers. The natural rubber or GR-S polymer content of prepared master batch stock may range from 75 to 90%, more or less. Natural rubber (smoked sheet) in chloroform was found to have only weak selective absorption, with a small shoulder at 270 to 290 mμ (E-0.25). On the other hand, GR-S exhibited selective absorption with distinct double maxima near 262 and 310 mμ, but the intensity of absorption (E-1.62 at 258 mμ) was relatively weak, as it was for natural rubber, when compared to the intensity of absorption of accelerators in Table I. Further investigation of the GR-S polymer proved the selective absorption observed here was due to a combination of the styrene molecule present in the GR-S (262 mμ) (*25*), and to the phenyl-2-naphthylamine antioxidant (*4, 26*).

Any absorption other than that of the accelerators in analysis of a master batch would be termed spectral interference. A method of correcting mathematically for the absorption of GR-S

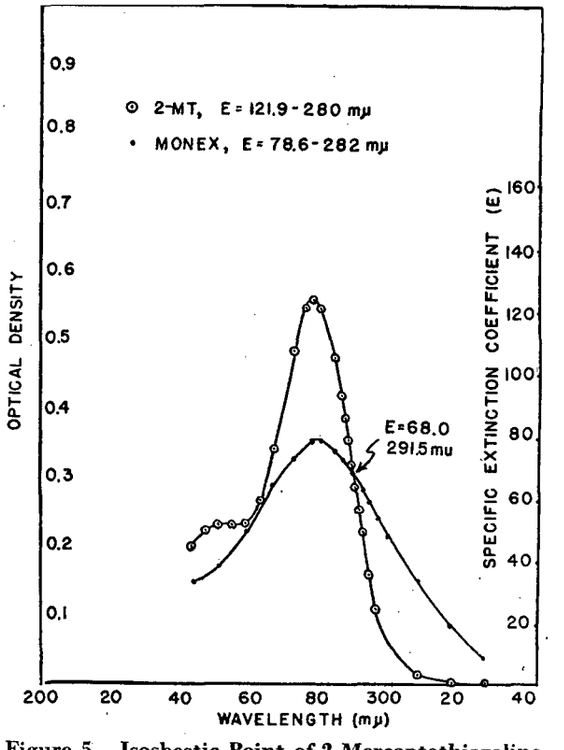

Figure 5. Isosbestic Point of 2-Mercaptothiazoline and Tetramethylthiuram Monosulfide in Chloroform

polymer in determination of phenyl-2-naphthylamine content (*4*), and a description of mathematical methods of eliminating various types of spectral interference have been published (*22*, *30*). As investigation showed such corrections were small with the particular samples of elastomers used here, they were neglected. However, in certain cases it may be necessary to apply mathematical corrections for interference in a master batch analysis.

Table II. Analysis of Single-Accelerator Master Batch Stocks

Accelerator and Elastomer	% as Milled	% Found	Stability of Solutions: Time standing, hours	% in day-light	% in dark	Maximum % drop
Captax in natural	25	24.1	2.5	22.4	..	−1.7
		24.8		..	24.4	−0.4
Captax in GR-S	10	9.9	..	..	..	...
		10.1				
Altax in natural	10	9.6	2.5	9.5	..	−0.1
		9.6		..	9.7	+0.1
Tuads in natural	25	24.2	No change in absorption on 1-hour standing in dark			
		24.1				
2-MT in GR-S	10	10.5	..	..	..	...

Data for Precision and Speed of Analysis

Samples placed in 50-ml. Erlenmeyer flasks into which 50 ml. of chloroform are pipetted. This represents practical technique for routine analysis of master batches. 10% 2-MT in GR-S gave following percentages: 10.7, 9.8, 10.5, 10.4, 10.6, and 10.2, an average of 10.4% with mean deviation of ±0.3%.

SINGLE-ACCELERATOR MASTER BATCH STOCKS

All master batches were mixed on a small laboratory rubber mill with smooth-faced rolls 2.5 inches in diameter by 6.5 inches long. The elastomer was first softened by milling, then the powdered accelerator was added with continued milling until a homogeneous stock was obtained.

It was desired to use as small a sample as convenient to facilitate rapid solution, and to eliminate need for a second dilution, thereby saving time and solvent, while still obtaining a concentration that allows spectrophotometric measurement well within the limits of the linear optical density range between 0.3 and 1.6.

The disadvantages of handling such small samples have been enumerated (*1*). Where the sample is thoroughly mixed in rubber and weighed as one piece, loss of sample is no concern, as it may be with weighing of powders. The method of preparing master batches by milling in rubber is an excellent example of factors tending to make a good dispersion and a homogeneous sample. Weighing errors are of greater importance with the small sample, but in this case some accuracy was deliberately sacrified to increase speed of analysis.

Procedure. A 4- to 8-mg. sample was cut from the prepared master batch stock and weighed accurately to 0.1 mg. with the 10-mg. rider of a double-beam balance. The weighed sample was transferred to a 100- or 50-ml. glass-stoppered volumetric flask. A half hour or so before spectral measurements were to be taken the flask was filled nearly full with C.P. reagent grade chloroform, placed in the dark under a laboratory bench, and shaken occasionally to speed solution.

Finally, the solution was diluted to volume with chloroform, shaken thoroughly, then poured directly into the 1-cm. quartz sample cell of the spectrophotometer without further dilution. Optical densities of this solution were recorded at the desired wave lengths, following the accepted procedure of instrument settings outlined earlier. Corrections were applied for cell transparency and cell thickness where necessary.

A more practical and entirely satisfactory solution procedure, eliminating the use of expensive and fragile glass-stoppered equipment, was followed in analysis of a 10% 2-mercaptothiazoline master batch. Here the weighed sample was placed in a 50-ml. Erlenmeyer flask, 50 ml. of chloroform were pipetted in, and the flask was closed with a cork stopper covered with aluminum foil.

The major part of master batches to be controlled for a rubber factory will probably be single-accelerator stocks of 10 to 25% accelerator content. Results of analysis of four different single-pigment master batches are reported in Table II, along with data on stability of chloroform solutions.

Accuracy. In every case but with 2-mercaptothiazole the percentages found were a little low, mainly because of loss of small amounts of accelerator on milling the pigment in the rubber. However, these differences were always less than 1% accelerator content with a 25% master batch and no greater than 0.4% for a 10% master batch.

It is clear that the 10% master batch can be analyzed with greater absolute accuracy than the 25% master batch. This same principle applies to analysis of two-component pigment mixtures. A difference of 5% in accelerator content of a powdered accelerator mixture will be a difference of only 1% when the same mixture is analyzed in a 20% master batch stock. Consequently, the application of the spectrophotometer to analysis of rubber master batches with acceptable absolute accuracy is an easier matter than analysis of two-component powdered accelerator mixtures. However, the relative accuracy measured as 20 ± 1% or 100 ± 5% would be the same. It is generally believed that relative accuracy is a better standard of measurement than absolute accuracy (*3*). In actual analysis the limits of a master batch would usually be expressed in terms of absolute accuracy—e.g., 20 ± 1% rather than relative accuracy of 20 ± 5%.

The accuracy required in routine master batch analysis cannot be made very exacting because these master batches are prepared in the factory where precision of weighing is limited. Analysis to ±1% of the specified percentage for a 25% master batch may be considered adequate control, and the spectrophotometer is capable of such accuracy, according to the data of Table II.

Precision. The two checks of a given sample agree very well in Table II, the greatest difference being 0.7% for 25% mercaptobenzothiazole master batch. Precision on analysis of six samples of 10% 2-mercaptothiazoline master batch was excellent. Excepting one sample of 9.8%, the remaining data fell within 0.3% of the average of 10.5%.

Speed of Analysis. Following the solution procedure outlined above for the 10% 2-mercaptothiazoline master batch, it is believed that as many as 100 samples of a single accelerator master batch could be readily analyzed in an 8-hour day by a single operator. Any saving in time over chemical methods must rest in the procedure for sample solution, and in time for measurement of the data. Weighing of sample and calculations may be expected to take about the same time for both the chemical and the physical ultraviolet spectrophotometric methods. When weights of the order of 4 to 8 mg. are used, solution of the sample can be effected in less than 20 minutes' standing in chloroform at room temperature. There is no particular need for more rapid solution, because samples may be weighed or spectral data recorded while other samples are dissolving. Shaking would speed up solution if needed.

Stability. The stability of master batch solutions in chloroform is seen to be adequate for routine analysis when samples are stored in the dark while solution is being effected, according to the data of Table II. It would be a preferable precaution from the standpoint of stability not to add chloroform to the weighed samples until 20 to 30 minutes before they are to be analyzed.

ANALYSIS OF TWO-COMPONENT ACCELERATOR MIXTURES

The usual accelerator master batch contains a single accelerator. However, the literature (*18*) frequently recommends the use of a secondary accelerator or "activator," usually added in a smaller amount than the primary accelerator, to modify or acti-

vate the action of the accelerator in vulcanization of the stock. If a rubber product formula calls for such a two-accelerator mixture, the separate master batches may be combined in the final stock to obtain the desired acceleration. This may be the essential procedure in a few cases to prevent preliminary accelerator interaction in the master batch while it is in storage. However, a practical saving in labor could be realized where a two-component accelerator master batch is prepared by adding both accelerators to the same master batch stock. Such a mixture may present a serious problem in rapid analysis for control purposes by chemical methods. Presence of one accelerator may interfere with the chemical determination of the other, giving high or low results. In the absence of such interference, it would still be necessary to analyze the same master batch by two different methods for the two-accelerator components.

Spectral methods offer the possibility of determining both accelerators in a single weighed sample, with a corresponding saving of time, and with no interference in analysis because of mixed acceleration.

Possible mixtures of two accelerators according to the literature (*18*) are: benzothiazyl disulfide acceleration with diphenylguanidine activation, and 2-mercaptothiazoline acceleration with tetramethylthiuram monosulfide activation. Another choice, to illustrate analysis of mercaptobenzothiazole in a mixture, was mercaptobenzothiazole and di-*o*-tolylguanidine. These particular accelerator mixtures, and percentages used, were chosen primarily to illustrate certain problems in spectrophotometric analysis of solutions containing two compounds. It is necessary to investigate these mixed powdered accelerator compounds prior to any attempt to analyze a master batch containing them.

The mixtures analyzed in this problem, other than those odd percentages weighed out directly, were prepared by weighing from 20 to 40 grams of each powdered accelerator and mixing them thoroughly by mechanical means until a homogeneous mixture was obtained, as shown by close agreement in results of analysis for a few portions of the mixture. The procedure followed was to pelletize the powdered mixture and weigh out from 5 to 20 mg. accurately on a balance sensitive to 0.1 mg. This was dissolved in 100 ml. of a solvent in a glass-stoppered volumetric flask, and an aliquot was taken (usually 5 ml.) for dilution to 50 ml. in a second glass-stoppered volumetric flask.

Except where a special study of light stability was being made, the solutions were shielded from direct light by an inverted cone of black paper around the body of the volumetric flask (stem exposed).

Isosbestic Points. Data of particular interest in analysis of mixtures are those recorded at an isosbestic point. Specifically, this is the term applied to the wave length where the intensity of absorption (as measured by E) for each component in a mixture is the same (*1*, *6*). By plotting the spectral curves on the same scale of specific extinction coefficient, the wave length at which their curves cross locates their isosbestic point of equal absorption intensity. This match point may be used to calculate the total per cent of materials present in a mixture, and may serve as a good quantitative check on other procedures of calculation.

The plot of 2-mercaptothiazoline and tetramethylthiuram monosulfide on the same specific extinction coefficient scale in Figure 5 illustrates this location of the isosbestic point, which is 291.5 mμ with E-68.0. In this case there is only one curve crossing and therefore one isosbestic point in the spectral region examined, but it is not uncommon for curves to cross at more than one wave length, though some mixtures may have no isosbestic point at all.

By plotting the data in a manner similar to that of Figure 5, the most acceptable isosbestic point of mercaptobenzothiazole and di-*o*-tolylguanidine was found to be at 287 mμ with E-14.6, and that of benzothiazyl disulfide and diphenylguanidine at 268 mμ with E-59.0. The actual use of these isosbestic points is described below.

The ultraviolet spectral curves of the above three mixtures of two components are illustrated in Figure 6.

Benzothiazyl Disulfide–Diphenylguanidine. The 60% benzothiazyl disulfide–40% diphenylguanidine pigment mixture shows a minor maximum at 266 to 270 mμ about midway between that of the diphenylguanidine (258 mμ) and benzothiazyl disulfide (274 mμ) alone. This illustrates the application of the spectrophotometer to analysis of a mixture where the wave length of maximum absorption for both components is not very far apart. Here the maxima are close enough so the overlapping additive absorption of the two components obscures the original shape of the single-accelerator curves, yielding the single maximum at 266 to 270 mμ.

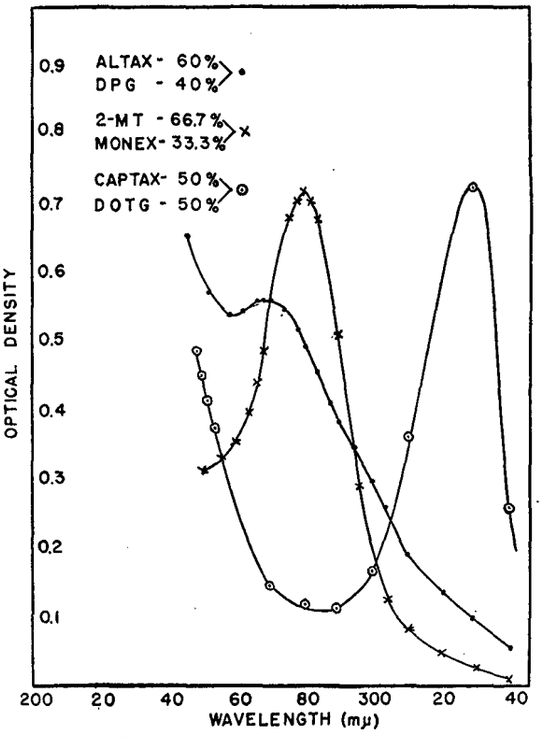

Figure 6. Two-Component Accelerator Mixtures in Chloroform

It is noted in Table III that the total per cent calculated at the isosbestic point wave length of 268 mμ agrees very well with the sum of per cent benzothiazyl disulfide and diphenylguanidine as calculated by equations. Where the total per cent is low, owing to fading of the benzothiazyl disulfide absorption, the per cent obtained at the isosbestic point is correspondingly reduced.

The wave lengths chosen for this calculation by the method of simultaneous equations (*11*, *13*) were those of the maxima at 258 mμ for diphenylguanidine and 274 mμ for benzothiazyl disulfide. According to the data of Table I, the difference in E at 274 mμ is only (62.1 − 53.5) or 8.6 units, while that at 280 mμ is (59 − 44) or 15 units, an increase of 70%. Though the 280 mμ wave length might appear to offer better accuracy because of this greater difference, it must be remembered from the curves of Figures 2 and 3 that both benzothiazyl disulfide and diphenylguanidine curves are changing rapidly in absorption (sloping side of maximum) at 280 mμ, and this is undesirable for greatest accuracy. The data of Table III show that either calculation of percentages at the maxima (258 mμ/274 mμ) or at 258 mμ/280 mμ) would give acceptable results. However, it seemed preferable to use the simultaneous equations set up at the maxima of both accelerators at 258 and 274 mμ.

Mercaptobenzothiazole–Di-*o*-tolylguanidine. The mercaptobenzothiazole maximum at 329 mμ is still present in the 50% mercaptobenzothiazole–50% di-*o*-tolylguanidine mixture, but the di-*o*-tolylguanidine maximum (252 mμ) is obscured by the rising curve of the more strongly absorbing mercaptobenzothiazole near the 252 mμ region in Figure 6.

Table III. Altax and DPG Accelerator Pigment Mixture in Chloroform

Sample	Weighed, % Concn.	% Found	% Deviation	Total % at 268 mμ (Isosbestic)
Calculation at 258 mμ and 274 mμ				
C				
Altax	60	55.4	−4.6	
DPG	40	42.5	+2.5	
		97.9		99.1
Calculation at 258 mμ and 280 mμ				
C				
Altax	60	54.0	−6.0	
DPG	40	44.1	+4.1	
		98.1		99.1

Stability Standing in Daylight Shielded with Black Paper Cone (Calculations at 258 and 274 mμ)

		Fresh Solution, %	After 20-Min. Standing, %	Change, %	% at 268 mμ Before	% at 268 mμ After
C						
Altax	60	55.4	56.8	+1.4		
DPG	40	42.5	39.7	−2.8		
		97.9	96.5		99.1	98.3

The greater the difference in intensity of absorption at the wave lengths chosen for analysis, the greater the potential accuracy of spectral methods. Here an opportunity is offered to analyze a mixture where the maxima are widely separated but where the difference in the intensity of absorption at one maximum (252 mμ) might prove inadequate for accurate analysis. The data of Table I show an average E at 252 mμ for mercaptobenzothiazole of 46.7 and for di-*o*-tolylguanidine of 50.9, which is only 4.2 units difference.

The possibility presents itself of determining di-*o*-tolylguanidine at 274 mμ near the minimum of mercaptobenzothiazole absorption, instead of at the 252 mμ di-*o*-tolylguanidine maximum, as the difference in E at 274 mμ amounts to 16.9 units, or four times that at 252 mμ. The data of Table IV for the mercaptobenzothiazole–di-*o*-tolylguanidine mixture calculated by simultaneous equation technique indicate that there is little difference between equations set up at 252 and 329 mμ or at 274 and 329 mμ, but the latter have been used in most of the calculations. The usual results totaled greater than 95%, but low recoveries for di-*o*-tolylguanidine were obtained in one series of samples.

As the data of Table I show, di-*o*-tolylguanidine absorption at the wave length of mercaptobenzothiazole maximum absorption (329 mμ) is small. It is so low relative to the strong mercaptobenzothiazole absorption that it can be neglected for all practical purposes, and mercaptobenzothiazole may then be determined rather accurately at 329 mμ without correction for di-*o*-tolylguanidine present. If the mercaptobenzothiazole absorption is then subtracted from that of di-*o*-tolylguanidine at 274 mμ, the absolute per cent di-*o*-tolylguanidine may be readily calculated by this single approximation. This represents a special case of calculation by successive approximation (*14*).

Because there are only two components present, the di-*o*-tolylguanidine content could be estimated here by difference from 100%, or by difference from total per cent determined at the isosbestic point. The data for this procedure of calculation by difference from 100% are listed in one column of Table IV. In this case it was the most accurate way to determine di-*o*-tolylguanidine content, because mercaptobenzothiazole absorption at 329 mμ was relatively stable, whereas di-*o*-tolylguanidine absorption apparently faded somewhat in chloroform solution.

The relative per cent composition of a two-component accelerator mixture or a master batch stock can be rapidly determined without weighing the sample by use of the ratio of absorption at one wave length relative to that at an isosbestic point. Thus per cent di-*o*-tolylguanidine was determined in the di-*o*-tolylguanidine–mercaptobenzothiazole mixture by the ratio of optical densities at 274 mμ and the isosbestic point at 287 mμ. This calibration curve showed a linear relationship between concentration and ratio of absorption (varying from 0.79 for 100% mercaptobenzothiazole to 1.88 for all di-*o*-tolylguanidine), yielding results even closer to correct di-*o*-tolylguanidine content of the powdered accelerator mixture than the data obtained by the simultaneous equation procedure.

Table IV. Captax and DOTG Accelerator Pigment Mixture in Chloroform

Sample	Weighed, % Concn.	% Found	% Deviation	Total % at 287 mμ (Isosbestic)	DOTG, % by Ratio 329/274 mμ	DOTG, % by Ratio 274/287 mμ
Calculations at 252 and 329 mμ						
A						
Captax	50	48.9	−1.1		R-4.16	R-1.312
DOTG	50	47.8	−2.2		49.5	50.5
		96.7		94.5		
Calculations at 274 and 329 mμ						
A						
Captax	50	48.7	−2.3			
DOTG	50	46.8	−3.2			
		95.5		94.5	....	.....
C						
Captax	69.3	67.6	−1.6		R-7.38	R-1.029
DOTG	30.7	25.4	−5.3		27.5	26.4
		93.0		94.3	(estimated)	
E						
Captax	31.8	30.4	−1.4		R-2.35	R-1.508
DOTG	68.2	61.1	−7.1		66.7	67.5
		91.5		90.5		

Results of Other Methods of Calculation

	Successive Approximation, %	Difference of % Captax from 100%	Difference of % Captax from Total at 287 mμ
C			
Captax	67.8		
DOTG	25.4	32.2 DOTG	26.5 DOTG
	93.2		
E			
Captax	30.4		
DOTG	61.1	69.6 DOTG	60.1 DOTG
	91.5		

The better accuracy for di-*o*-tolylguanidine content by this ratio method might be explained by the fact that any fading of di-*o*-tolylguanidine absorption in chloroform at 274 mμ may also take place proportionally at the isosbestic point of 287 mμ. Therefore, the percentages determined by ratio will not be as greatly affected when the isosbestic point is used, as when the 274 and 329 mμ absorption is applied in the method of simultaneous equations. It has been mentioned in the literature (*1*) that if something effects a change in curve shape of a solution, it may still be possible to obtain useful data where there is an isosbestic point that may be used for the measurement, giving a point that is more or less independent of the past history of a solution.

An absorption ratio at a maximum and minimum of a single component present in a mixture may be used for calculation of one component. This technique was applied to the mercaptobenzothiazole–di-*o*-tolylguanidine mixture at 329 mμ maximum and 274 mμ minimum of mercaptobenzothiazole. In this case the variation of ratio with concentration was more complex than was ratio at an isosbestic point. Though a plot of the relationship was not a straight-line function here, a satisfactory calibration curve was set up empirically.

Analysis of specially weighed out mixtures of more and less than the 50% di-*o*-tolylguanidine used above indicates that this

spectral procedure will detect any appreciable change in accelerator content of the mixture and determine the extent of that change.

More careful shielding of the samples from light, or other modifications of technique, would doubtless improve the accuracy for spectral analysis of this pigment mixture. But the degree of accuracy and precision obtained here would probably prove to be adequate for control of 10 to 25% mercaptobenzothiazole–di-*o*-tolylguanidine in a master batch.

Table V. 2-MT and Monex Pigment Mixture in Chloroform

Sample	Weighed, % Concn.	% Found by *E* Value	% Deviation	% 2-MT by Ratio 280/291.5 mμ	Total % at 291.5 mμ
A		*E*-106.6			(Isosbestic)
2-MT	66.7	65.3	−1.4	..	...
Monex	33.3	34.7[a]			
B		*E*-107.9			
2-MT	66.7	68.1	+1.4	..	...
Monex	33.3	31.9[a]			
E		*E*-107.2			
2-MT	66.7	66.7[a]	0.0	..	...
Monex	33.3	33.3			
Av. 2-MT content		65.5			
Av. *E* value of six samples		*E*-106.7			
		Varying Percentages of 2-MT and Monex			
G					
2-MT	39.7	39.5	−0.2	43	98.2
Monex	60.3	60.5[a]			
H					
2-MT	66.7	70.5	+3.8	71	100.0
Monex	33.3	29.5[a]			
I					
2-MT	69.1	69.2	+0.1	74	98.0
Monex	30.9	30.8[a]			

[a] % 2-MT found by *E* value of mixture, and % Monex calculated by difference from 100%.

2-Mercaptothiazoline–Tetramethylthiuram Monosulfide. In the particular case of the 66.7% 2-mercaptothiazoline and 33.3% tetramethylthiuram monosulfide mixture is a unique, but entirely practical, possibility of analyzing a two-component mixture where both accelerators have a single absorption maximum at nearly the same wave length. The small 2 mμ difference between the maximum of 2-mercaptothiazoline (280 mμ) and tetramethylthiuram monosulfide (282 mμ) results in a single strong maximum at 280 mμ for the mixture. However, there is a wide difference of 43.7 units in intensity of absorption at 280 mμ for 2-mercaptothiazoline (*E*-121.9) and tetramethylthiuram monosulfide (*E*-78.2) and a method of analysis based on this difference was devised.

The absorption of 2-mercaptothiazoline and tetramethylthiuram monosulfide at 280 mμ is additive, as long as the mixture complies with Beer's law, so the *E* value at 280 mμ will rise progressively from the low of 78.2 for tetramethylthiuram monosulfide to a high of 121.9 for 2-mercaptothiazoline as the per cent 2-mercaptothiazoline is increased in the mixture. A straight-line relationship was established by calculations using the above-mentioned upper and lower limits in *E* value at 280 mμ, and a calibration curve was plotted.

Analysis of a 2 to 1 mixture of 2-mercaptothiazoline and tetramethylthiuram monosulfide using this procedure based on *E* value showed relatively good precision and accuracy, with an average of 65.5% 2-mercaptothiazoline. When specially weighed out mixtures of low (39.7%) and high (69.2%) 2-mercaptothiazoline were analyzed using this method, the data obtained proved this technique satisfactory for determining varying percentages in the mixture with acceptable accuracy. The isosbestic point (291.5 mμ) located in Figure 5 is observed to give near 100% summation of 2-mercaptothiazoline and tetramethylthiuram monosulfide concentration in Table V. The absorption at this point may be used with the ratio technique at the maximum (280 mμ) to determine the relative per cent 2-mercaptothiazoline in the tetramethylthiuram monosulfide, just as a ratio was used for the di-*o*-tolylguanidine–mercaptobenzothiazole mixed accelerators. Percentages obtained graphically by *E* value appear more accurate than the ratio data, but both techniques yield acceptable results.

In the case of 2-mercaptothiazoline and tetramethylthiuram monosulfide, the difference in *E* value at 280 mμ is 43.8 units (122.0 − 78.2), while with mercaptobenzothiazole and di-*o*-tolylguanidine at 274 mμ it was only 16.9 units (27.5 − 10.6). Consequently, the accuracy of analysis of the 2-mercaptothiazoline–tetramethylthiuram monosulfide mixture is proportionally greater than that of the di-*o*-tolylguanidine–mercaptobenzothiazole mixture, even though the range of ratio variation for the 2-mercaptothiazoline–tetramethylthiuram monosulfide mixture (1.15 to 1.79) is smaller than that for the di-*o*-tolylguanidine–mercaptobenzothiazole (0.79 to 1.88).

Accelerator Identity. In chemical procedures the accelerators of a certain type, such as diphenylguanidine and di-*o*-tolylguanidine of the guanidine class, may be determined in a master batch by the same chemical method (*7*). Thus, the possibility exists of a mixed identity not detected in normal chemical control, particularly when two-component acceleration is present in a factory prepared stock. The spectrophotometer is well adapted to detection and rapid identification of any such mixed master batches that may occur. A check of the ratio of absorption at two or three selected wave lengths would serve to determine rapidly whether the sample being analyzed contains the type of acceleration specified.

TWO-COMPONENT ACCELERATOR MASTER BATCH STOCKS

The accuracy and precision with which two-component accelerator master batches may be analyzed are not so good as those for the single accelerator master batch, but they appear to be adequate for control purposes, as illustrated in Table VI.

The procedure for analysis is the same as for the single-accelerator master batch stock. Calculations are made by the same equations established for two-component powdered accelerator mixtures. One difference is for the 2-mercaptothiazoline–tetramethylthiuram monosulfide master batch calculated by graphical methods using the *E* value. This technique cannot be used alone with a master batch without a check point, because a high or low per cent might mean one of three things. An increase in total per cent analysis under these conditions could mean an actual increase in total per cent 2-mercaptothiazoline–tetramethylthiuram monosulfide, an increase in the 2-mercaptothiazo-

Table VI. Master Batches of Two-Component Accelerator in Natural Smoked Sheet

Accelerator	% Components in Master Batch		% Found		Total % at Isosbestic
60% Altax	Altax	12.0	11.0	*E* = 59.0 at 268 mμ	
40% DPG	DPG	8.0	A 8.7		
	Total	20.0	19.7		20.0
			11.5		
			B 8.8		
			20.3		20.6
50% Captax	Captax	10.0	10.2	*E* = 14.6 at 287 mμ	
50% DOTG	DOTG	10.0	A 9.6		
	Total	20.0	19.8		20.7
			10.6		
			10.7		
			21.3		22.0

% Components in Master Batch	Total at 280 mμ (*E* = 106.7)	Total at 291.5 mμ (*E* = 68.0)	% 2-MT Based on *E*	% 2-MT by Ratio 280/291.5 Absorption
2-MT 13.3	20.2	20.2	13.3	R-1.572; 13.2
Monex 6.7	20.2	20.0	12.8	R-1.562; 12.8
Total 20.0	20.2	20.3	13.1	R-1.564; 13.0
	19.8	19.7	..	....

line content in the mixture, or both possibilities together. This problem can be solved easily enough by calculating the total per cent at the isosbestic point (291.5 mμ) and determining the 280 mμ absorption of the mixture as before, then calculating the 2-mercaptothiazoline content relative to the isosbestic point total. A second possible procedure is use of the ratio of Table V for the 2-mercaptothiazoline content, pertaining to ratio of 280 mμ maximum and 291.5 mμ isosbestic point.

MATHEMATICS

Beer's law was followed for all the compounds under investigation with acceptable accuracy over an optical density range of 0.3 to 1.6, so direct mathematical treatment could be used with no need for calibration curves (*3*, *21*).

The per cent of an accelerator in a single-component master batch stock may be found by determining the specific extinction coefficient of the master batch at the wave length selected for analysis and calculating the proportion of master batch absorption relative to the specific extinction coefficient of the commercial accelerator itself, as given in Table I. This assumes it is not necessary to allow for absorption of ultraviolet radiation by the elastomer present (spectral interference), though this could be done mathematically by accepted procedures if advisable (*4*, *22*, *30*).

The more complex mathematics of a multicomponent mixture can be conveniently classed as determining either absolute or relative concentrations. The preferred method of calculating absolute concentration of a two-component mixture is by use of simultaneous equations set up to represent the absorption (optical density) at the wave lengths chosen for analysis (*11*, *13*). The method of determinants is believed to be more direct, rapid, and simple than an analytical procedure for solving the set of simultaneous equations algebraically, even for a two-component mixture.

The technique using difference in quantitative absorption (E) at the same wave length is best applied for absolute concentration where the maxima of both components are close together and their E values are appreciably different. Analyzing for per cent of one component by difference from 100% total is sometimes useful, but is indirect. A more acceptable and direct procedure for absolute analysis by difference is to determine total concentration at the isosbestic point.

The relative per cent composition of a two-component mixture can be rapidly determined by use of the absorption ratio or by difference in measured absorption. The elimination of weighing is the great advantage of relative analysis, which is capable of yielding excellent accuracy as well as speed. It is particularly useful where total concentration and sample composition are definitely known, as with a known two-component powdered accelerator mixture, where it can be used for very rapid estimation of relative concentrations. This technique is limited in analysis of a master batch, because absolute content of the accelerator in rubber is not determined.

When there is no isosbestic point, an absorption ratio at a maximum and minimum or some other wave length of a single component present may be used. The ratio relationship may not always be linear, which makes possible calculation by direct interpolation, but a satisfactory calibration curve can be set up empirically.

A method for determining relative composition utilizing the difference in optical densities at selected wave lengths of a two-component mixture has been described (*32*). Though this offers advantages with liquid samples, weighing cannot be eliminated with a solid master batch using this technique where total concentration must be known or kept constant. Use of a two-coordinate optical density plot as a calibration curve (*13*) offers an entirely graphical procedure particularly useful for cases of Beer's law failure. Relative analysis by the internal standard technique commonly applied in spectroscopic methods does not appear practical in master batch analysis for several reasons.

SUMMARY

Different solvents were found to be capable of altering the spectral absorption of an accelerator in both selectivity (curve shape) and intensity. Chloroform was chosen as the spectral solvent most suitable for rapid master batch solution and analysis. The photosensitivity of certain accelerators in this solvent was controlled by shielding solutions from direct daylight, which reduced fading of ultraviolet absorption to an acceptable level. There was no evidence of any interaction in chloroform of mixed accelerators studied.

The spectral absorption of seven different commercial accelerators in chloroform was bound to be characteristically different in selectivity and intensity of absorption. Work with several single-accelerator and two-component accelerator master batches, each presenting a somewhat different problem in analysis, showed that the spectral method of quantitative analysis was applicable in every case. Interference by absorption of the elastomer in the master batch was found to be relatively negligible. The final analytical procedure proved to be rapid, precise, and accurate enough for use in routine control of master batch stocks.

The method of simultaneous equations was favored for calculation of absolute per cent composition, while a procedure utilizing ratios of absorption at different wave lengths, one preferably at an isosbestic point, rapidly determined the relative composition of unweighed powdered accelerator mixtures and master batch stocks.

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Retraction Test for Serviceability of Elastomers at Low Temperatures

O. H. SMITH, W. A. HERMONAT, H. E. HAXO, AND A. W. MEYER
United States Rubber Co., Passaic, N. J.

Elastomer vulcanizates progressively stiffen as the temperature is lowered. Additional stiffening, due to crystallization, may occur as exposure to low temperatures is prolonged. The available methods of testing the low temperature flexibility of rubber and rubberlike materials do not reveal the losses in flexibility caused by crystallization except by using prolonged storage at low temperatures. A retraction test employing large deformations, which greatly increases the rate of crystallization, has been developed. This test rapidly gives a temperature index correlating with the stiffness of elastomer vulcanizates after storage at low temperatures, and can be used to measure the merit for low temperature applications of both crystallizable and noncrystallizable elastomers. This test in conjunction with conventional (room temperature) tests has been used successfully to study the low temperature performance of Hevea, GR-S, Paracril, and polybutadiene vulcanizates along with vulcanizates of many experimental elastomers. Correlation of results with cold compression set and hardness after low temperature storage has been excellent and substantiates the usefulness of the test.

WHEN the temperature is lowered, the flexibility of a polymer decreases as the second-order transition temperature is approached. The decrease in flexibility is caused by increased internal viscosity. This phenomenon is called the retarded elastic effect or viscoelastic effect. Additional decreased flexibility may be caused by first-order transition effects (crystallization) in polymers having a structure of sufficient regularity. When usefulness of elastomers at low temperatures is evaluated, both effects should be measured whenever possible.

A retraction test has been developed which rapidly gives a temperature index that correlates with the ultimate stiffness of elastomer vulcanizates at low temperatures. Ultimate stiffness includes increased modulus due to viscoelastic and first-order transition effects if present. The large deformation employed in this test (250% elongation) causes rapid appearance of the stiffening due to crystallization in polymers having a regular structure. The test is based upon the T-50 test (*4*). Various similar pieces of apparatus have been designed which yield the same type of data. Yerzley *et al.* (*8*) applied such a test to neoprene and stated that it was inadequate. More recently, Svetlik (*7*) applied a technique to the study of elastomers using only 50% test elongation. The test described in this paper uses 250% initial elongation and differs from other tests also in the method of analyzing the data.

APPARATUS AND TESTING METHOD

The retraction tester consists of an apparatus that permits the measurement of the elongation of 2-inch T-50 samples (*1*) (60-gage) at all times during a run. The front view in Figure 1 shows the holder with the samples inserted; three of the samples are unstretched and three are stretched ready for insertion in the cooling bath. Wire leads (piano wire), attached to the samples by means of hooks, pass through binding posts which permit the samples to be anchored at any elongation. Strings attached to the ends of the wire leads pass over small pulleys at the top of the instrument. The free ends of the string are attached to small counterweights. A scale graduated in 0.1 inch is inserted behind the leads. Attached to the leads are disk-shaped indicators to enable the length of the sample to be read.

The over-all view in Figure 1 shows the apparatus standing in an unsilvered Dewar flask which is contained in a wooden frame. This frame, which was built to act as a convenient stand for the apparatus and additional insulation, is filled with glass wool and held in place with a sheet of polyethylene. A window in the frame permits the reading of a totally immersed thermometer. The Dewar flask contains a stirrer and a heating element connected to the house current through an autotransformer to maintain a proper heating rate.

The procedure is based upon the background material described in the following sections. A 2-inch T-50 sample (60-gage) of the vulcanizate under test is placed in the hooks, stretched 250% (from 2 to 7 inches), and locked in the stretched position by turning the thumb nut on the binding post. The rack containing the stretched samples is placed in a methanol bath, which had been cooled to −70° C. by dipping into it dry ice contained in a cylindrical wire cage. The stretched samples are conditioned for 10 minutes. The thumb nuts are released, allowing the samples to retract freely. The temperature of the bath is then raised 1° C. per minute by means of the heating coil. The length of each sample is measured at 2° intervals. The bath is agitated throughout the test.

The temperatures at which the sample retracts 10, 30, 50, and 70% of the original elongation are called TR10, TR30, TR50, and TR70, respectively. These values give an adequate picture of

the low temperature behavior. Retraction values (TR10, TR30, TR50, TR70) are computed from the data by the following formula:

$$\% \text{ retraction} = 100\left(1 - \frac{L_T - L_0}{L_e - L_0}\right)$$

where L_e = over-all length of sample in stretched condition at start of test, L_T = length at observed temperature, and L_0 = length in unstretched condition.

For example, when L_T is 6.5 inches at temperature T and the sample was stretched from 2 to 7 inches at the start of the test, the per cent retraction equals

$$100\left(1 - \frac{6.5 - 2}{7.0 - 2}\right) \text{ or } 10\%$$

The temperature at which this occurs is the TR10 value.

TYPICAL DATA

Figure 2 contains typical retraction curves of Hevea and GR-S 10 vulcanizates containing 50 parts of carbon black, accelerators, and 2 parts of sulfur. GR-S 10 does not crystallize and thereby yields a smooth retraction curve. However, Hevea has a strong tendency to crystallize, which causes an irregular retraction curve. The retraction values were obtained from these curves and recorded in Table I. In the remainder of the paper retraction curves are not given; only the retraction values are presented—that is, the TR10, TR30, TR50, and TR70 values.

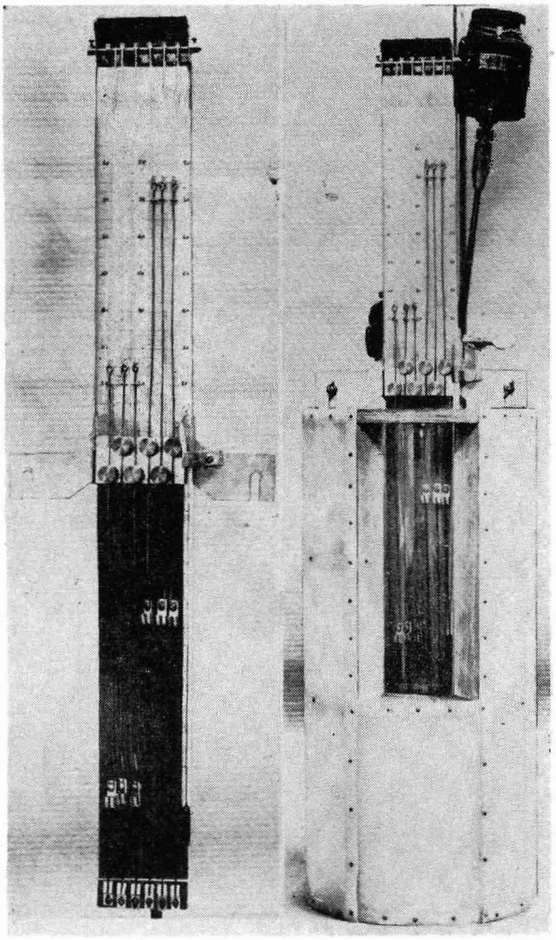

Figure 1. Retraction Apparatus

Left. Sample holder
Right. Over-all view

The TR10 value indicates the low temperature merit of the elastomer prior to low temperature storage. In this respect, this value is similar to the T_{10} (3) value obtained when using the torsion modulus test. This measurement is influenced by viscoelastic effects and very little by crystallization. Because crystallization does influence low temperature properties greatly, especially after low temperature storage, additional criteria such as TR70 must be used to obtain a complete picture of the low temperature behavior of elastomers.

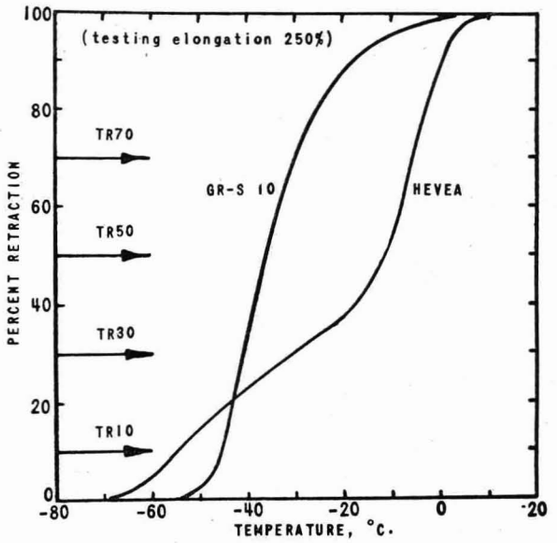

Figure 2. Typical Retraction Data on GR-S 10 and Hevea

The TR70 value indicates the low temperature merit of the elastomer after a long period of low temperature storage. This measurement is influenced by both viscoelastic effects and crystallization, thereby giving a measure of ultimate stiffness. Hevea has a TR70 value of −5.0° C. and GR-S 10 has a TR70 value of −28.7° C. This indicates that upon storage at low temperatures Hevea would eventually become less flexible than GR-S 10, especially under static stress. Thus, higher cold compression set was found for Hevea than for GR-S 10.

Table I. Retraction Values of GR-S 10 and Hevea from Curves in Figure 1

	TR10	TR30	TR50	TR70
GR-S 10, ° C.	−45.4	−40.3	−35.5	−28.7
Hevea, ° C.	−54.2	−29.6	−11.1	−5.0

Effect of Elongation on Retraction Values. The sample is purposely given a large deformation to induce rapid crystallization upon cooling. The retraction values of the Hevea and GR-S 10 vulcanizates previously described were measured at various testing elongations. It was found that increasing the testing elongation of Hevea (above 100%) caused a sharp rise in the TR70 value, which begins to level off at 200% elongation (see Figure 3). This rise is caused by the presence of crystallization during the test. Near maximum effect is reached at 250% testing elongation. Increasing the testing elongation of GR-S 10 causes the TR70 value to decrease sharply until 200% is reached; thereafter larger deformations cause little further decrease. The TR10 values are influenced only slightly by changes in testing elongation.

Because little change in the retraction values occurs on increasing the elongation above 250%, this elongation was adopted as

standard. Many experimental vulcanizates break in the apparatus when greater elongations are used. Using 250% elongation is desirable from that standpoint also.

Testing Hevea Gum Stocks. The use of 250% testing elongation has been found adequate in every case except Hevea gum vulcanizates. Using the standard testing procedure of 250% testing elongation for Hevea vulcanizates containing less than 40 parts of carbon black will give misleading results. In Figure 4 are data showing the effect of both testing elongation and concentration of carbon black on TR70. Using 250% testing elongation for a pure gum Hevea gave a TR70 of −56.8° C., whereas a testing elongation of 400% gave a TR70 of −1.8° C. Therefore, elongations of 400% should be used for Hevea gum stocks.

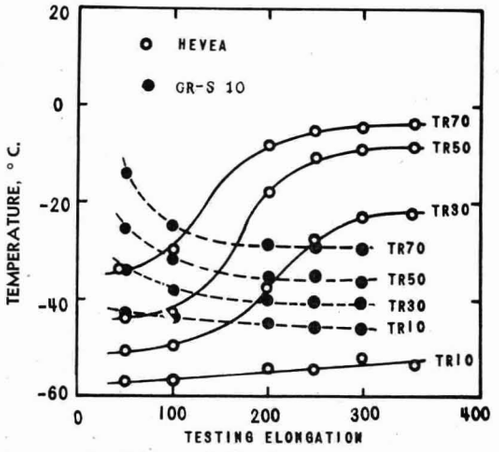

Figure 3. Effect of Testing Elongation upon Retraction Values

Effect of Low Temperature Storage upon TR70. Normally, compounded Hevea crystallizes slowly. When the storage temperature is varied from the optimum, −25° C., the crystallization may appear more slowly. The same effect occurs in GR-S elastomers having low styrene content when polymerized at low temperatures; the optimum for such elastomers is close to −45° C. It is also known that the application of stress to a sample increases the rate of crystallization. The result of these tendencies depends upon the selected storage temperature and degree of stress imposed upon the sample. Under the conditions of this test crystallization appears rapidly.

Table II. Effect of Storage at −55° C. upon TR70 of Vulcanizates of Elastomers

Polymer	Time of Storage, Hours	Storage Temperature, ° C.	TR70, ° C.
Hevea	0		−5.4
	71	−55	−4.0
GR-S 10	0		−29.8
	73	−55	−29.7
B/S, 90/10, 41° F.	0		−30.4
	73	−55	−29.1
Polybutadiene, 41° F.	0		−17.0
	73	−55	−15.0
Polybutadiene, 77 °F.	0		−34.1
	72	−55	−32.4

It is believed that 250% testing elongation creates a condition which induces nearly maximum crystallization during the short time of the test. This was verified by storing the retraction samples at 250% elongation at −55° C. Without warming them, the samples were plunged to −70° C. in the retraction bath placed in the cold box and a subsequent run was made. The TR70 determined by this procedure and the normal procedure is compared in Table II. If storage at low temperature has increased the crystallization in a sample, its TR70 should be at a higher temperature. Only small changes due to storage were observed. The magnitude of the change in TR70 due to storage is close to the accuracy of the test. Therefore, these changes are not considered significant, but they are indicative of the high degree of crystallinity obtained in this test.

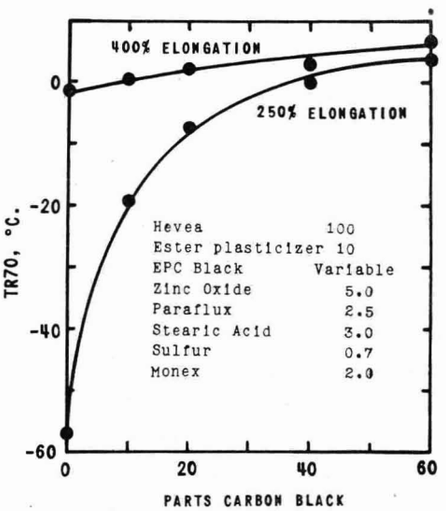

Figure 4. Effect of Carbon Black on TR70 of Hevea Vulcanizates

The retraction values for several standard elastomers are contained in Table III. The TR10 values indicate that Hevea and Butyl will be the most flexible materials at low temperatures when no storage is encountered. The order of merit as described by TR10 would be polybutadiene (41° F.), GR-I, Hevea, GR-S 10, GR-S (41° F.), neoprene, and Paracril. However, if each material is stored at low temperatures to allow crystallization to take place, the flexibility of polybutadiene (41° F.), Hevea, and neoprene would be greatly reduced and the order of merit would become: GR-S 10, GR-S (41° F.), GR-I, polybutadiene (41° F.), Paracril, Hevea, and neoprene as indicated by the TR70 values.

LOW TEMPERATURE FLEXIBILITY OF BUTADIENE-STYRENE POLYMERS

The TR70 of several polybutadiene vulcanizates made at various temperatures has been measured. Table IV contains the

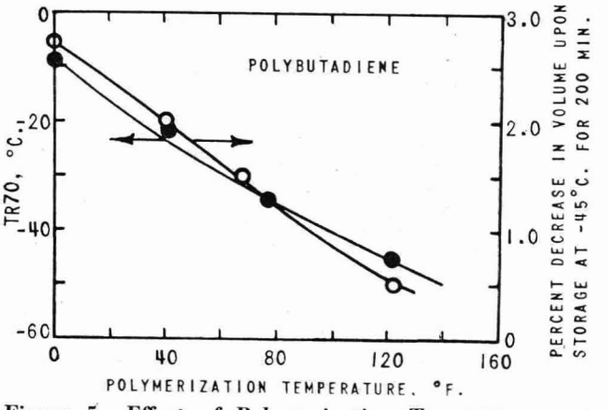

Figure 5. Effect of Polymerization Temperature on TR70 and Extent of Crystallization for Polybutadiene

Table III. Retraction Values of Standard Elastomers

(° C.)

Rubber	TR10	TR30	TR50	TR70
GR-S 10	−45.4	−40.3	−35.5	−28.7
GR-S (41° F.)	−45.1	−40.2	−34.8	−27.8
GR-I	−54.3	−41.8	−31.9	−23.7
Polybutadiene (41° F.)	−59.9	−41.3	−29.3	−21.8
Paracril 26NS90	−27.5	−22.5	−17.8	−12.0
Hevea	−54.2	−29.6	−11.1	−5.0
Neoprene	−40.4	−35.2	−24.6	+3.6

Compounded with 50 parts of black using standard curing methods.

Table IV. Type Formula

Polymer	100
EPC channel black	50
Zinc oxide	5
Paraflux	5
Stearic acid	1.5
Sulfur	2.0[a]
MBT[b]	1.5
DPG[c]	0.4[d]

Cure 45 minutes at 292° F.

[a] Except where otherwise specified.
[b] Mercaptobenzothiazole.
[c] Diphenylguanidine.
[d] Varied to equalize rate of cure.

vulcanization details. It was found that decreasing the temperature of polymerization caused the TR70 to rise (see Figure 5). Decreasing the temperature of polymerization from 122° to 0° F. raised the TR70 from −45.4° to −8.9° C. This change can be explained by understanding the accompanying structural changes. Hart and Meyer (*5*) found by infrared absorption studies that the temperature of polymerization increased the structural regularity, as predicted through x-ray analysis by Beu *et al.* (*2*) and through dilatometer work by Lucas (*6*). Some dilatometer data obtained at the General Laboratories are presented in Figure 5. It may be assumed that the decrease in volume accompanying low temperature storage is due to crystallization and this decrease is a measure of the crystallization. It can be easily seen that increasing crystallization due to low temperature polymerization raises the retraction value TR70.

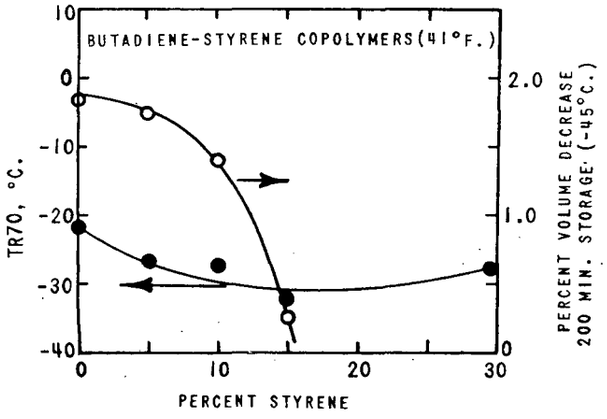

Figure 6. Effect of Styrene on TR70 and Extent of Crystallization

Copolymerization of butadiene with comonomers having high second-order transition temperatures generally results in polymers having poorer low temperature properties. Figure 6 contains a plot of TR70 against the styrene content in vulcanizates of butadiene-styrene copolymers made at 41° F. The compounding formula is presented in Table IV. Figure 6 shows that increasing the styrene content to 15 or 20 parts actually improves the ultimate flexibility of the vulcanizates at low temperatures. Even a polymer containing 29% styrene has a lower TR70 than polybutadiene. These apparent discrepancies can be explained by determining the crystallization in these polymers. These results are also presented in Figure 6. The addition of 15 parts of styrene as a comonomer reduces the crystallization to a very low quantity for 41° F. copolymers. Here the decrease in volume with storage at −45° C. is almost negligible. Reducing crystallization by introducing a comonomer, styrene, improves the TR70 despite the rise in second-order transition temperature which accompanies this procedure.

Usually ultimate stiffness is difficult to measure using ordinary test methods, because increasing the comonomer not only decreases the amount of crystallization but also decreases the rate at which it appears. This effect is exaggerated when the polymer is in the compounded condition. The cure in these polymers produces large effects upon their low temperature characteristics.

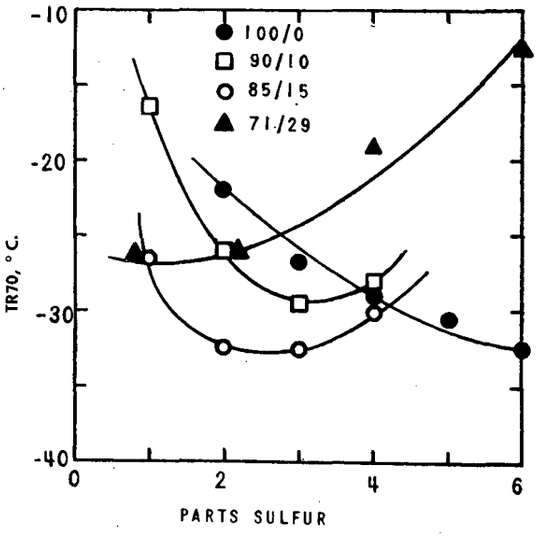

Figure 7. Effect of Sulfur on TR70 of Butadiene-Styrene Copolymers Made at 41° F.

Figure 7 contains the TR70 values of vulcanizates of butadiene-styrene copolymers made at 41° F. with various states of cure. The compounding formula is contained in Table IV. A sharp improvement is noted in the low temperature properties, as indicated by TR70 values of polybutadiene with increasing cure. However, increasing cure for GR-S (29% styrene) made at 41° F. produces vulcanizates of poorer low temperature flexibility. Increasing cure reduces crystallization, thereby improving the TR70 of polybutadiene, and also reduces flexibility owing to the increased number of sulfur cross links. Because there is no crystallization in GR-S made at 41° F., increasing cure produces poorer TR70 values. The plot of TR70 versus cure will show a minimum for a polymer having an intermediate styrene content. The minimum TR70 indicates the state of cure at which crystallization has been arrested. A polymer containing 10 parts of styrene requires a higher cure than the polymer containing 15 parts of styrene to obtain this minimum.

CORRELATION OF TRIO WITH T_{10}

It was stated above that the TR10 value was a function of the viscoelastic effect but not of crystallization. The TR10 can therefore be used as a figure of merit for low temperature flexibility of crystalline elastomers for dynamic applications, or for noncrystalline elastomers under all low temperature conditions. This figure of merit will be similar to the T_{10} value obtained when testing low temperature flexibility with a torsion modulus apparatus.

The TR10 and T_{10} values were measured for vulcanizates of butadiene-isoprene, butadiene-styrene, butadiene-acrylonitrile, and butadiene-isoprene-acrylonitrile. Because exact knowledge of the composition of these elastomers is not essential to the understanding of the test, this information has not been included. Table V contains compounding data. Formula A was used for butadiene-isoprene and butadiene-styrene, whereas Formula B was used exclusively for polymers containing acrylonitrile. In Figure 8 is a plot of the TR10 value versus the T_{10} value of these vulcanizates. The points form a straight line. The order of low temperature merit of the polymers given by the TR10 is therefore similar to that given by T_{10}.

This correlation of TR10 with T_{10} is more general than is indicated in Figure 8. In addition to the polymers mentioned above, Hevea, polyisoprene, terpolymers of butadiene and isoprene with styrene, aryl acrylates, and vinylpyridine also have the same correlation. Variation of sulfur from 1 to 4 parts, addition of plasticizers (ester or hydrocarbon), and changes in carbon black content do not alter the relationship. It is believed, therefore, that the TR10 can be used reliably as a measure of the low temperature flexibility of elastomer vulcanizates where low temperature storage is not encountered and for noncrystalline vulcanizates.

TR70 AS A MEASURE OF COMPRESSION SET

Both TR70 and compression set (measured according to Navy Department Specifications 33-R-9) were measured on a series of experimental stocks, which contained 15 parts of ester plasticizer and 40 parts of Philblack O. It was found that the TR70 could be used as a index of compression set, because stocks having equal TR70 values have equal compression set values. Naturally, as the TR70 values decrease the compression set values also decrease.

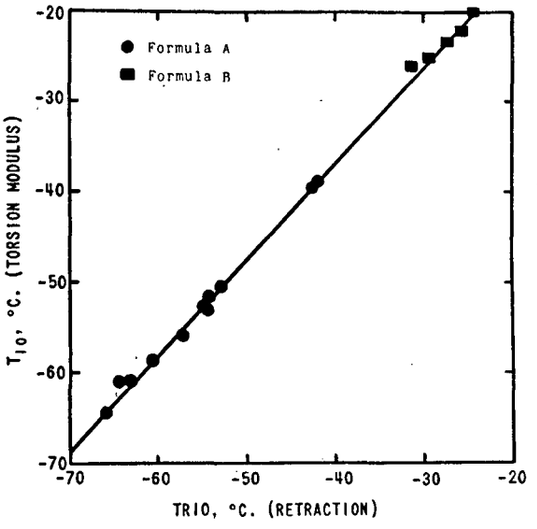

Figure 8. T_{10} *vs.* TR10 of Butadiene-Styrene, Butadiene-Isoprene, and Butadiene-Acrylonitrile Vulcanizates

If the TR70 of these stocks is plotted against their compression set (at −35° and −45° C.) smooth curves are developed (see Figures 9 and 10). The retraction test can now be used to estimate the compression set of vulcanizates. This function is important, because the retraction test can be performed in 45 minutes. Storage (48 to 96 hours) is necessary when the compression set values are measured.

At −35° C., but not at −45° C., the compression set values of Hevea stocks lie on the curve. This apparent discrepancy is caused by crystallization, which takes place less rapidly at −45° C. than at −35° C. Under the conditions of the compression set test, apparently maximum crystallization was not obtained at −45° C. Hevea stocks therefore, when stored at −45° C. for 96 hours, yield compression set values that are too low. If the storage time of the compression set test were increased sufficiently, the compression set values would eventually rise to the value indicated by its TR70 in Figure 10.

Table V. Type Formula

	A	B[a]
Polymer	100	100
EPC black	50	50
Zinc oxide	5	5
Paraflux	5	...
Stearic acid	1.5	1.5
Sulfur	2	1–2
MBT[b]	1.5	...
DPG[c]	0.4[d]	...
MBTS[e]	...	1.5

Cure 45 minutes at 292° F.

[a] Used exclusively for polymers containing acrylonitrile.
[b] Mercaptobenzothiazole.
[c] Diphenylguanidine.
[d] Varied to equalize cure.
[e] Mercaptobenzothiazyl disulfide.

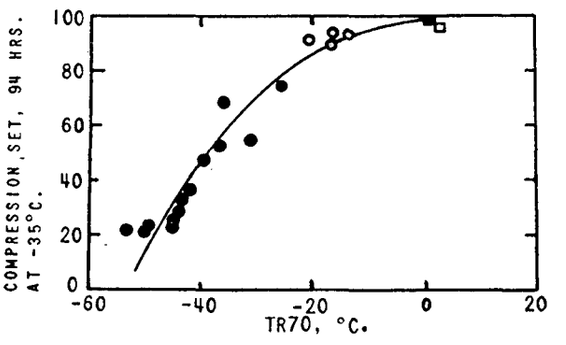

Figure 9. Compression Set *vs.* TR70

● Butadiene-styrene copolymers
○ Perbunan
■ Hevea (50 parts channel black)
□ Hevea (gum)

CORRELATION OF TR70 WITH HARDENING IN COLD STORAGE

TR70 has also been correlated with hardening in cold storage (Figures 11 and 12). Both tread stocks containing 50 parts of easy processing carbon black and 2 parts of sulfur and gasket stocks containing 15 parts of ester plasticizer, 40 parts of Philblack O, and 0.7 parts of sulfur were included in this correlation.

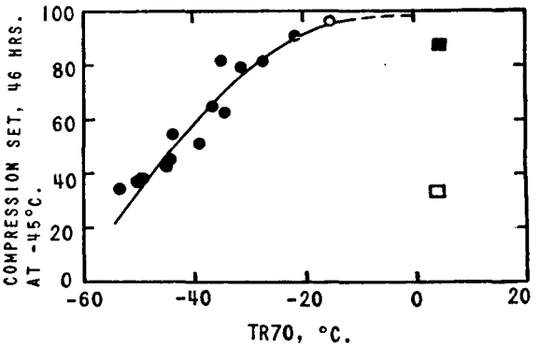

Figure 10. Compression Set *vs.* TR70

● Butadiene-styrene copolymers
○ Perbunan
■ Hevea (50 parts channel black)
□ Hevea (gum)

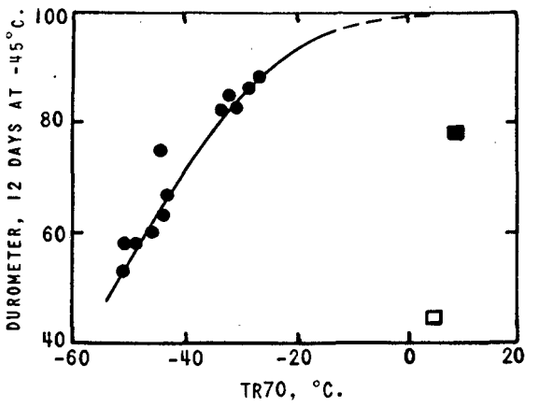

Figure 11. Hardness after Storage at −45° C. *vs.* TR70

● Butadiene-isoprene-styrene co- and terpolymers
■ Hevea (50 parts channel black)
□ Hevea (gum)

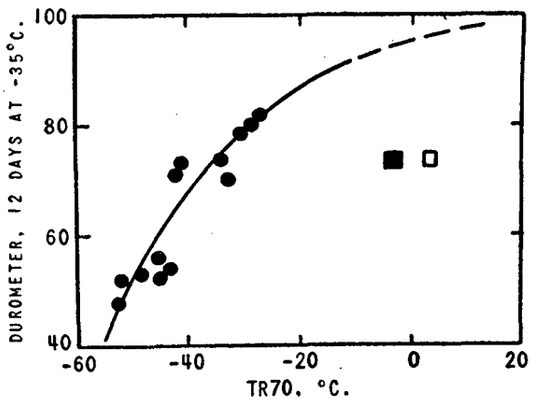

Figure 12. Hardness after Storage at −35° C. *vs.* TR70

● Butadiene-isoprene-styrene co- and terpolymers
■ Hevea (50 parts channel black)
□ Hevea (gum)

Most of the stocks were based on butadiene-styrene copolymers polymerized at 41° and 122° F., respectively, and contained various proportions of styrene. Tread stocks based on Hevea, butadiene-isoprene copolymers, butadiene-isoprene-styrene terpolymer, and a gasket stock containing no black based on Hevea were included. Durometers were measured at intervals over 12-day periods at −35° and −45° C., respectively. Hardness of all the stocks with the exception of the Hevea stocks correlated fairly well with TR70 at both −35° and −45° C.

Hardness of the Hevea stocks did not correlate with TR70 at either temperature. The smoked sheet tread compound attained approximately equivalent hardness at −35° and −45° C. The smoked sheet gasket compound was about as hard as the tread compound when stored for 12 days at −35° C., but when stored for a similar period at −45° C. the gasket compound hardened very little whereas the tread compound became even harder than at −35° C.

The difference between the correlation of the Hevea compound with TR70 at −35° C. in the hardness test and the compression set test discussed previously can be explained by assuming less crystallization in the hardness test due to the absence of strain which increases tendency to crystallize.

ACKNOWLEDGMENT

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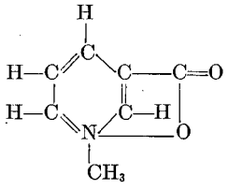
Determination of Trigonelline in Coffee

R. G. MOORES AND DOROTHY M. GRENINGER, *General Foods Corp., Hoboken, N. J.*

Trigonelline represents about 5% of the soluble solids in coffee beverage. It contributes to coffee flavor and aroma, and may have important physiological properties. An easy and reliable method for determining trigonelline was needed in the study of coffee composition and flavor development. In the new method, trigonelline is purified by chromatography on a solid adsorbent column, and is measured by ultraviolet absorption spectrophotometry. This procedure is more reliable and convenient than earlier methods. The method will be useful in further studies on the composition and processing of coffee, as well as in physiological studies involving closely related compounds such as nicotinic acid.

THE presence of trigonelline in green coffee was first reported by Polstorff in 1909 (*21*), and later verified by Gorter (*6*). It is the methylbetaine of nicotinic acid with the structural formula:



Trigonelline represents about 5% of the water-soluble portion of roasted coffee. It has a bitter taste about one fourth that of caffeine. Trigonelline may be considered the source of pyridine found in coffee aroma. Hughes and Smith (*9*) found pyridine

and nicotinic acid among the decomposition products of trigonelline when it was heated in a sealed tube at 150° to 220° C.

The structure of trigonelline suggests the possibility of its demethylation in vivo to nicotinic acid, the antipellagra factor. However, the physiological properties reported in the literature are conflicting and lead to no certain conclusions regarding its dietary role (*3, 7, 14, 18*).

A reliable analytical procedure for trigonelline is important because of its contribution to coffee flavor and aroma and its possible biological significance.

ANALYTICAL PROCEDURES FOR TRIGONELLINE

Several methods for the determination of trigonelline have been described in the literature (*4, 12, 19, 22, 23*). Their deficiencies are discussed briefly in the following paragraphs.

Precipitation Methods. The trigonelline method of Nottbohm and Mayer (*19*) involves precipitation of trigonelline with iodine from a chlorogenic acid and caffeine-free coffee extract, and titration of the iodine complex in alcohol with thiosulfate. The iodine precipitation requires a high concentration of trigonelline for quantitative results. The method can be applied only to green coffee, because the iodine salt does not crystallize readily in the presence of roasted coffee constituents.

Methods have been reported for the determination of bismuth based on the precipitation of alkaloid iodobismuthates (*5, 16*). Trigonelline was found to precipitate quantitatively, on an equal molar basis, with this reagent. This method gave good results on green coffee, but satisfactory precipitation could not be obtained on roasted coffee.

A modification of the Slotta and Neisser procedure (*23*) was used previously for trigonelline analysis in this laboratory. This method, which involves a phosphotungstic acid precipitation of the trigonelline from a lead-clarified water extract and alkaline oxidation of the regenerated trigonelline, is inconvenient and is subject to errors that limit its reliability.

Colorimetric Methods. The measurement of trigonelline would be simplified by conversion to a soluble colored product, but none of the conversion techniques tried in this work were quantitative. Colorimetric methods suggested in the literature for the estimation of pyridine ring compounds depend upon the formation of Schiff's bases from the corresponding glutaconaldehydes (*12, 13*). However, this reaction cannot be applied directly to the pentavalent nitrogen in trigonelline.

Sarett, Perlzweig, and Levy (*20, 22*) found that alkaline hydrolysis of trigonelline in the presence of a source of ammonia yielded a substance that develops a color with cyanogen bromide and amines; the trigonelline presumably had been converted to nicotinic acid. However, they found that this conversion amounted to only 70%. Recent work by Huff (*8*) has shown that the conversion of trigonelline to nicotinic acid is not quantitative; under the most favorable conditions the nicotinic acid isolated was only 30% of the theoretical yield.

Kodicek and Wang (*12*) and Fox, McNeil, and Field (*4*) measured trigonelline by the color of the compound formed when benzidine or dianisidine is added to the glutaconaldehyde formed on alkaline hydrolysis.

Preliminary analyses in this laboratory on pure trigonelline solutions by a slight modification of the dianisidine method were unsatisfactory. The time for development of maximum color varied from 14 to 37 minutes. Reproducibility of maximum readings was poor; when 4 mg. of trigonelline were hydrolyzed, maximum absorbancy measurements varied from 0.724 to 1.072. The addition of urea as a buffer in the neutralization did not improve reproducibility. With a pH range of 6.35 to 8.47 at the time of the addition of the color reagents, the highest absorbancy reading was obtained at pH 8.2. After 24 hours' standing, solutions of hydrolyzed trigonelline gave less color with the reagent, and absorbancy readings were not proportional to concentration.

Spectrophotometric Properties of Trigonelline. Following the development of satisfactory spectrophotometric methods for determining chlorogenic acid (*17*) and caffeine (*11*) in coffee, absorption measurements were applied to trigonelline. Trigonelline in water solution has a maximum absorption at 264.5 and a minimum at 240 mμ (Figure 1).

Absorbancy of aqueous trigonelline solutions is proportional to concentration and is constant over a pH range of 4 to 8. Other substances in coffee have absorption in this region—e.g., caffeine, chlorogenic acid, proteins, etc. No chemical reagent could be found that selectively separated trigonelline without itself absorbing light in the same region.

The unsatisfactory results obtained in the use of known color and precipitation reagents indicated the need for an entirely different approach for the purification and estimation of trigonelline. Several adsorbents, such as Zeokarb H, X-L clay, Lloyd's reagent, zeolite, Amberlite IR-100, and Filtrol, remove trigonelline from pure solution. The first three adsorbents contain soluble materials which absorb light at 265 mμ and cannot be removed by any reasonable amount of washing. Both the percolate and the ammonia eluate from zeolite contain nontrigonelline material which absorbs light at 265 mμ. Amberlite IR-100 can be washed fairly free of the interfering materials, but elution of the trigonelline cannot be made quantitative. With Filtrol, adsorption of the trigonelline from an alcohol-water solution and elution with ammonium hydroxide are quantitative (Table I). These solvents do not remove any material that interferes with spectrophotometric measurements.

Standard Trigonelline Samples. Two samples of trigonelline were used in this work. One sample was prepared by the methylation of nicotinic acid, using the method of Winterstein and Weinhagen (*25*). It was identical in ultraviolet absorption and iodine equivalent with a reference standard obtained from the S.M.A. Corp., Chagrin Falls, Ohio. The reference standard had a nitrogen content of 10.27% by the Dumas method. The theoretical value is 10.22%. At 264.5 mμ, the wave length of

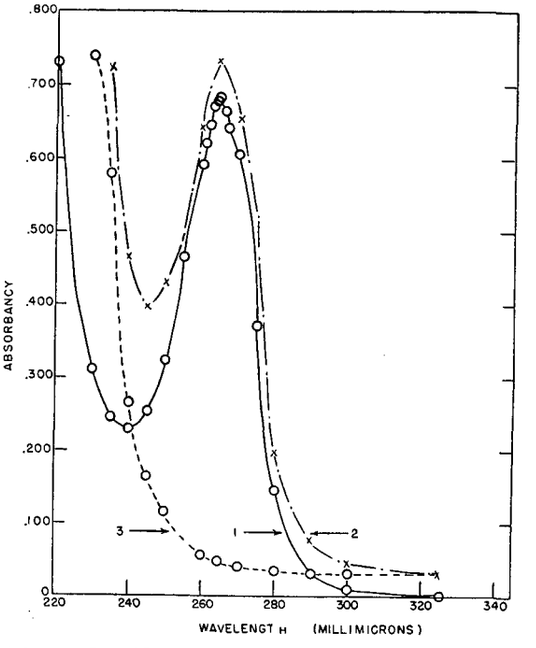


Figure 1. Absorption Curves for Trigonelline before and after Filtrol Absorption

1. Pure trigonelline
2. Trigonelline after adsorption and elution from Filtrol
3. Blank

Table I. Recovery of Trigonelline by Adsorption and Elution from Filtrol

Sample Description	Trigonelline Recovery in Eluate by Ultraviolet Absorption, %
Pure trigonelline	104.0
	102.1
	100.2
	104.2
	101.7
	105.4
Mixture of trigonelline, caffeine, and chlorogenic acid	101.2
	103.2
	101.9
	99.7
	98.4
	99.5
	97.3
	100.0
Mixture of trigonelline, caffeine, chlorogenic acid, and nicotinic acid	98.0[a]
	101.2[a]
Pure trigonelline added to green coffee extract	100.8
	100.0
Pure trigonelline added to roasted coffee extract	98.9
	98.9

[a] Corrected for nicotinic acid on basis that 100% of total nicotinic acid was in eluate.

maximum absorption, both samples of trigonelline had an $E^{1\%}_{1\text{ cm.}}$ of 297.

ULTRAVIOLET ABSORPTION METHOD FOR TRIGONELLINE

Reagents and Equipment. Ethyl alcohol, 95 and 50% by volume.

Sulfuric acid, 2% by volume in 95% ethyl alcohol.

Ammonium hydroxide, 3% by volume, 30 ml. of 28.7% ammonium hydroxide per liter.

Potassium permanganate, 1% by weight in water.

Sodium sulfite, 5% by weight in water.

Potassium ferrocyanide, 106 grams of $K_4Fe(CN)_6.3H_2O$ dissolved in water and diluted to 1 liter.

Zinc acetate, 219 grams of $Zn(C_2H_3O_2)_2.2H_2O$ and 30 ml. of glacial acetic acid dissolved in water and diluted to 1 liter.

Acetic acid, C.P. glacial.

Filter paper, Green's fluted No. 488½; 15-cm., Whatman No. 5.

Celite, No. 545, and analytical (Johns-Manville Corp., New York, N. Y.).

Filtrol, Super Filtrol (Filtrol Corp., Los Angeles, Calif.). Prepare adsorption mixture by thoroughly mixing 2 parts of Celite No. 545 and 1 part of Filtrol.

Extraction and adsorption tubes. Attach 80-mm. lengths of 6-mm. glass tubing to the bottom of 18 × 150 mm. test tubes.

Beckman quartz spectrophotometer, Model DU.

Sample Preparation. Coffee samples are prepared for analysis by grinding and flaking as for chlorogenic acid (*17*).

Extraction of Green and Roasted Coffee. Weigh accurately 2 grams of sample and mix with 4 grams of Celite No. 545. Transfer to an extraction tube containing a glass wool plug and Celite No. 545 filter bed. Place the extraction tube in a 500-ml. filter flask connected to a vacuum line. Percolate 200 ml. of 50% ethyl alcohol through the sample at a uniform rate of about 7 ml. per minute, transfer the extract to a 200-ml. volumetric flask, and make to volume.

Adsorption and Elution of Trigonelline. Prepare a Filtrol adsorption column by placing a glass wool plug in the bottom of the tube and covering with a Celite No. 545 bed about 10 mm. thick. On top of the Celite bed, place about 6 grams of the 2 to 1 Celite-Filtrol mixture. Tap the tube, connect to a 500-ml. filter flask, and apply vacuum to compress the column. Percolate through the tube 75 ml. of 2 *N* sulfuric acid in about 10 minutes.

Transfer 100 ml. of the coffee extract to a 250-ml. separatory funnel equipped with a rubber stopper or ground-glass joint to fit the top of the adsorption tube. After the acid wash of the column is complete, connect to the adsorption tube the separatory funnel holding the coffee extract. Draw the coffee extract through the Filtrol column at a uniform rate of about 5 ml. per minute. As soon as the extract is drawn through, wash the column successively with 50 ml. of 50% ethyl alcohol, 50 ml. of 2% sulfuric acid in 95% ethyl alcohol, and 50 ml. of 95% ethyl alcohol. Some liquid should be retained on top of the adsorption column at all times to avoid channeling.

Remove the filter flask containing the trigonelline-free extract and wash solutions, and replace it with a clean 250-ml. filter flask. Elute the adsorbed trigonelline by percolating through the column 150 ml. of ammonium hydroxide. Transfer the eluate to a 200-ml. volumetric flask and dilute to volume with water. The time for the elution should be about 20 minutes, making the total time for adsorption, washing, and elution about 90 minutes.

Measurement of Trigonelline in Green Coffee. Transfer 50 ml. of the eluate to a 100-ml. volumetric flask and dilute to volume. Measure the absorbancy (absorbancy or A_s = optical density) of the diluted eluate at 264.5 and 325 mμ. Subtract the absorbancy at 325 from the absorbancy at 264.5 mμ and from this difference calculate the trigonelline content, using an $E^{1\%}_{1\text{ cm.}}$ value of 297.

Measurement of Trigonelline in Roasted Coffee. Place 50 ml. of the eluate in a 100-ml. volumetric flask, add 10 ml. of potassium permanganate solution, mix, and let stand 10 minutes. Add 3 ml. of sodium sulfite solution with stirring, then 1.5 ml. of acetic acid, and finally titrate with sulfite solution to the disappearance of the manganese dioxide precipitate. Make to volume and filter through No. 5 Whitman paper, discarding the first portion. Measure the absorbancy at 264.5 mμ.

Place a separate 50-ml. aliquot of the eluate in a 100-ml. volumetric flask, and add about 25 ml. of water and 1 ml. of acetic acid. Add 5 ml. of zinc acetate solution and mix; then with swirling add 5 ml. of potassium ferrocyanide solution. Make to volume and shake thoroughly. After 3 to 5 minutes filter through a dry folded paper, such as Green's No. 488½. Discard the first 5 to 10 ml. and collect enough to fill an absorption cell. Measure the absorbancy at 264.5 mμ. Subtract this from the previous absorbancy reading and calculate the trigonelline content using this corrected figure.

DISCUSSION

Sample Preparation of Trigonelline Extraction. The importance of proper sample preparation has been demonstrated in the current work. Essentially complete cell rupture, as provided by flaking, is necessary for reliable analysis of plant material such as coffee. Extraction of trigonelline with 50% ethyl alcohol is more satisfactory than extraction with 95% ethyl alcohol or water. Extraction with 95% ethyl alcohol is extremely slow, while water extracts percolate very slowly through Filtrol columns.

Adsorption and Elution with Filtrol. Under proper conditions of percolation, washing, and elution, the Filtrol column can be used to separate trigonelline quantitatively from caffeine and chlorogenic acid in pure solutions (Table I). In the concentrations normally present in coffee extracts, caffeine is adsorbed on the column but not eluted by the ammonia. Chlorogenic acid is adsorbed on Filtrol and is eluted with ammonia. However, when the adsorbent is washed with acid before percolation of the alcohol extract, the adsorption of chlorogenic acid is reduced, and washing with acid–ethyl alcohol after the percolation effectively elutes the small amount of chlorogenic acid adhering to the column. Washing with acid–ethyl alcohol does not remove the trigonelline, but does remove some of the ammonia-elutable substances present in roasted coffee extracts which absorb light at and below 325 mμ.

Hughes and Smith (*9*) reported that only 1 to 3% of the trigonelline loss on roasting could be attributed to nicotinic acid formation. The present work substantiates their data by finding little or no nicotinic acid when trigonelline was roasted alone or in mixtures of coffee constituents. These observations are based on results of alkaline iodine titrations. Nicotinic acid does not react with iodine under the conditions used for trigonelline analysis. Nicotinic acid is adsorbed and eluted along with trigonelline in the proposed method and has an absorption maximum near 265 mμ.

Trigonelline in Green Coffee. The trigonelline content of green coffee by the spectrophotometric method is about 10% lower than that obtained by the original Slotta-Neisser procedure (Table II). The absorption figures have been substantiated by applying the Slotta-Neisser iodine titration to the ammonia-free eluates from the Filtrol column. Good agreement between the

absorption measurements and the iodine titrations indicates better selectivity in the present isolation technique.

The correction for the absorption reading at 325 mμ is used as the alternative to a blank sample correction (Figure 2). This measured absorption is apparently caused by light-scattering substances washed from the Filtrol column. In the present method the effect of these substances on the absorbancy measurements is sufficiently constant between 265 and 325 mμ to make feasible use of the latter reading as a correction for the sample absorbancy at 264.5 mμ. This correction amounts to no more than 0.05 absorbancy unit.

Absorption measurements on the eluates before and after treatment with zinc ferrocyanide substantiate further the specificity of the present method for trigonelline in green coffee. This reagent precipitates trigonelline quantitatively from water solutions without interfering with the light absorption at 264.5 mμ. The difference between the total absorption and the absorption removed from the eluates by zinc ferrocyanide treatment is equal to the blank (Figure 2), so that the trigonelline content is essentially the same when calculated using a blank correction or on the basis of absorption removed by ferrocyanide treatment (Table II).

Table II. Trigonelline Content of Green Coffee[a]

		Spectrophotometric Method		
	Slotta-Neisser Method, %	Total A_s of eluate at 264.5 mμ, %	Total A_s minus blank[b], %	A_s removed by precipitation[c], %
Santos	1.32	1.22	1.17	...
	1.33	1.25	1.21	...
		1.36	1.25	1.25
		1.30	1.23	1.23
		1.29	1.22	1.18
		1.29	1.22	1.20
Av.	1.33	1.29	1.22	1.22
Guatemalan	1.11	1.02	0.98	...
	1.11	1.03	0.99	...
		1.02	0.98	...
		1.02	0.98	...
		1.09	1.05	1.00
		1.09	1.05	0.99
Av.	1.11	1.05	1.01	1.00
Colombian	1.06	1.00	0.95	...
	1.06	1.01	0.96	...
		0.98	0.94	...
		0.99	0.94	...
		1.14	1.03	1.03
		1.15	1.03	1.03
Av.	1.06	1.05	0.96	1.03

[a] All results expressed on dry weight basis.
[b] Trigonelline calculated from total absorbancy, A_s, of eluate at 264.5 mμ minus absorbancy at 325 mμ.
[c] Trigonelline calculated from difference between total absorbancy of eluate and that remaining after zinc ferrocyanide treatment.

Trigonelline in Roasted Coffee. Roasting plant materials generally increases their chemical complexity and magnifies analytical difficulties. Coffee is no exception, and extensive experimental work was required before the spectrophotometric method could be applied to roasted coffee. Experiments were designed to study the effect of roasting on trigonelline alone and in the presence of other coffee constituents, in order to define the nontrigonelline materials which absorb light at 264.5 mμ.

Heating pure trigonelline with 12% added water in a sealed glass tube at 180° to 187° C. for 63 minutes causes a significant change in its light absorption behavior. The alcohol extract of the roasted trigonelline has no peak near 265 mμ, but has fairly high absorption in the entire spectral region from 240 to 325 mμ. Iodometric titration directly on the alcohol extract after evaporation shows an apparent trigonelline loss of 83%. This alcohol extract of the roasted trigonelline contains no trigonelline by the ultraviolet absorption method. Because analyses on roasted coffee do not show such a drastic change in the nature of the trigonelline, the presence of other materials in coffee must prevent this complete degradation of trigonelline during roasting.

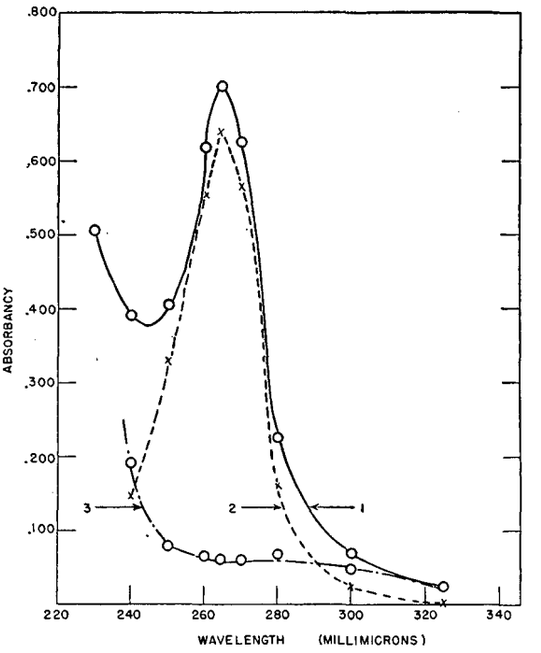


Figure 2. Absorption Curves for Trigonelline in Green Coffee Extracts

1. After adsorption and elution on Filtrol, no treatment
2. Absorption removed from eluate by permanganate-ferrocyanide treatment
3. Eluate after permanganate-ferrocyanide treatment

A mixture containing chlorogenic acid, sucrose, green coffee dialyzate, caffeine, and potassium phosphate buffer at pH 5.85 was roasted with and without added trigonelline.

Direct spectral absorbancy readings at 264.5 mμ on alcohol extracts of the roasted products indicated only a 10% loss of trigonelline on roasting. When the alcohol extracts were carried through the adsorption-elution with Filtrol, spectrophotometric measurements indicated a loss of 68% of the trigonelline. Iodometric titration of these Filtrol eluates showed a trigonelline loss of 69%, which substantiates the ultraviolet measurement.

Because nicotinic acid does not react with iodine under these conditions, the agreement between the ultraviolet and iodometric measurements indicates that little if any trigonelline has been converted to nicotinic acid during the roasting. The absorbancy at 264.5 mμ in the Filtrol eluates from roasted simulated coffee extracts containing no trigonelline was negligible, indicating no interference from the other constituents. High transmittancy in all the eluates from 285 to 325 mμ indicated that no light-scattering materials were interfering. It is apparent that when trigonelline is roasted pure, or in a mixture of soluble green coffee constituents, substances are formed which absorb light at 264.5 mμ, but these substances are largely separated from the trigonelline in the adsorption-elution.

Direct ultraviolet absorption on the ammonia eluates from Filtrol columns (Table III) showed an apparent increase in trigonelline during the roasting of coffee. This apparent increase and the shape of the absorption curve indicated that the absorbancy of the roasted coffee eluates was not due entirely to trigonelline and that further purification was necessary. Because not all of these interfering substances absorb at 325 mμ, this absorbancy cannot be used as a correction for roasted samples.

Potassium permanganate has been used as a purification reagent in methods for determining caffeine in coffee (*1*, *2*, *11*, *15*). This reagent in alkaline, neutral, or acid solution was found to have no effect on the ultraviolet absorption of pure trigonelline.

Permanganate-sulfite treatment of roasted coffee eluates decreases appreciably the 265 mμ absorbancy and largely eliminates the discrepancy between the absorbancy curves of the eluate and pure trigonelline solution.

A zinc ferrocyanide precipitation of trigonelline has been used to determine the specificity of the absorbancy of the permanganate-sulfite treated eluates. When zinc acetate–potassium ferrocyanide treatment is applied to roasted coffee eluates, there remains some absorbancy in the region 265 to 325 mμ (Figure 3). The residual absorbancy is the same when this clarification is applied directly to the eluate as to the permanganate-sulfite treated solution. It is more convenient to use the eluates before permanganate-sulfite treatment.

Although the interfering material has not been identified, it is not trigonelline, and the residual absorbancy has been subtracted as a blank in the trigonelline determination for roasted coffee. The trigonelline analysis in roasted coffee depends upon the specificity of the Filtrol adsorption and elution, the removal of major interfering materials by mild oxidation, and selective precipitation of the trigonelline.

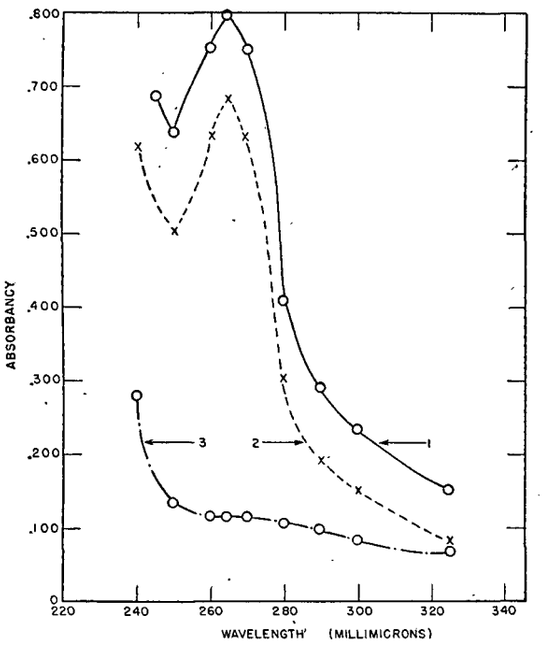


Figure 3. Absorption Curves for Trigonelline in Roasted Coffee Extracts

1. **Eluate after permanganate treatment**
2. **Absorption removed from permanganate treated eluate by ferrocyanide treatment**
3. **Eluate after permanganate-ferrocyanide treatment**

Hughes and Smith (*9*, *10*) have reported that trigonelline is lost during roasting and that the amount lost varies with the time and temperature of roasting. In the present work the average decrease in trigonelline for three samples of coffee with an organic roasting weight loss of 9.7% was 39% by the Slotta-Neisser method and only 15% by the spectrophotometric method (Table III). This difference can be explained by the failure of the phosphotungstic acid to precipitate trigonelline completely from roasted coffee extracts. Apparently some of the products formed during coffee roasting interfere with this precipitation and also with precipitation by iodine or iodobismuthate. The spectrophotometric method, in which this interference is not a factor, gives a higher and more accurate measurement of trigonelline in roasted coffee.

Table III. Trigonelline Content of Roasted Coffee[a]

	Roasting Wt. Loss Excluding H_2O, % Green Coffee	Slotta-Neisser Method, %	Spectrophotometric Method: Total A_s at 264.5 mμ, %	Spectrophotometric Method: Total A_s minus blank[b], %	Spectrophotometric Method: A_s removed by precipitation[c], %
Santos	9.2	0.89	1.70	1.15	1.13
		0.89	1.53	1.03	1.16
					1.17
					1.19
Av.		0.89	1.61	1.09	1.16
Guatemalan	9.8	0.76	1.84	1.16	0.97
		0.74	1.84	1.16	0.94
					0.94
Av.		0.75	1.84	1.16	0.95
Colombian	10.2	0.72	1.74	1.12	0.92
		0.72	1.78	1.16	0.92
					0.92
Av.		0.72	1.76	1.14	0.92

[a] All results expressed on dry weight basis.
[b] Trigonelline calculated from total absorbancy of eluate at 264.5 mμ minus absorbancy at 325 mμ.
[c] Trigonelline calculated from difference between total absorbancy of eluate and that remaining after zinc ferrocyanide treatment.

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Determination of Penicillin G

Using C^{13} Isotope as a Tracer

JOHN T. CRAIG, J. B. TINDALL, AND MURRAY SENKUS, *Commercial Solvents Corp., Terre Haute, Ind.*

A precise method was developed for the determination of penicillin G in relatively impure samples of penicillin broth and crystalline samples. Labeled potassium benzylpenicillinate was enriched with respect to C^{13} at the carbon attached to the benzyl group. The enrichment in analytical samples was determined by hydrolysis of the penicillin to phenylacetic acid, followed by decarboxylation of the acid to carbon dioxide. The C^{13} content of the gas sample was determined in a mass spectrometer. The method of analysis should prove to be of great value in developing and improving process for the isolation of penicillin G. The analysis of a broth is of special significance in this connection, inasmuch as it reveals the amount of penicillin G per unit volume. A biological assay of broth can be obtained, but these data cannot be used to calculate the penicillin G content.

THREE chemical and physical methods have been developed for the determination of penicillin G in penicillin samples. In the gravimetric method, the material is isolated quantitatively as the *N*-ethylpiperidinium salt (*10*). In one spectroscopic method the penicillin G content is obtained by measuring its ultraviolet absorption in solution (*4*), while in another method it is obtained by measuring the infrared absorption of a crystalline sample (*2*).

These three procedures are the most precise methods that are available for the purpose at hand. However, their utility is limited, inasmuch as they are confined to relatively pure samples. In the evaluation of a recovery process, they can be used only for the analysis of final products, and they have proved to be indispensable for this purpose ever since industry started to produce crystalline penicillin. Obviously, differentiation between penicillins in each of the preceding steps is highly desirable, particularly when attempts are made to separate the penicillins during the recovery and to control the production of penicillin G in the broth through the use of precursors or through the selection of molds.

Several microbiological methods have been described for the differentiation between penicillins (*3*, *5*, *11*) in impure samples. In this laboratory the Thorn and Johnson method (*11*) has proved to be especially useful, inasmuch as it is particularly suited to the determination of penicillin G in samples ranging in purity from that found in broth to that in crystalline samples. The precision of this method is ±10%.

The method described herein is an isotope dilution method for the determination of penicillin G, using C^{13} as a tracer. Rittenberg and Foster (*9*) have developed an isotope dilution method with application to the determination of amino acids through the use of N^{15} and fatty acids using deuterium.

Labeled penicillin was prepared by using as a precursor in the fermentation phenylacetamide containing excess C^{13} at the amide group. The method has been applied to the determination of penicillin G in broth as well as in commercial crystalline samples.

EXPERIMENTAL

Microbiological Preparation of Labeled Benzylpenicillinic Acid. Eastman Kodak potassium cyanide containing 16 atom % C^{13} was converted to phenylacetonitrile according to the method of Adams (*1*). The nitrile was then hydrolyzed to phenylacetamide as described by Purgotti (*7*). To a medium suitable for the production of penicillin G was added 0.04% by weight of the amide. The mixture was sterilized and inocculated with *Penicillium chrysogenum* Wisconsin strain Q-176. The fermentation was allowed to proceed at 25° C. in 2-liter Erlenmeyer flasks agitated on reciprocal shakers. The broth was harvested after 5 days.

To each 3-liter portion of the C^{13} filtered broth were added 2 grams of pure potassium benzylpenicillinate in order to reduce the C^{13}/C^{12} ratio at the amide carbon to about 4/100, because it was calculated that this would be a suitable ratio for the labeled standard.

Isolation of Crystalline Labeled Potassium Benzylpenicillinate. Crystalline potassium benzylpenicillinate was isolated from the broth by way of crystalline benzylpenicillinic acid diisopropyl etherate, which has been reported by Trenner (*12*), with some modification. Any method for the isolation of crystalline benzylpenicillin from broth could be used at this point.

The etherate was converted to the potassium salt by dissolving in butyl acetate, then adding dropwise with good agitation 0.5% aqueous potassium hydroxide until the pH of the aqueous phase had reached 7.5. The aqueous layer was separated and dried from the frozen state. Crystallization of the solid product from *n*-butyl alcohol according to the method of Wintersteiner (*13*) gave 1.8 grams of crystalline potassium benzylpenicillinate, the labeled standard.

Several 3-liter portions of broth were processed to obtain a large quantity of labeled potassium benzylpenicillinate, followed by subsequent recrystallization of the composite material.

Isotope Concentration of Labeled Standard. A mixture consisting of about 1 gram of the labeled potassium benzylpenicillinate and 50 ml. of 4 *N* sulfuric acid was allowed to reflux for 2 hours. The solution was extracted with ethyl ether. The extract was distilled to remove the ethyl ether. To the residue were added 15 ml. of warm petroleum ether to dissolve the crude phenylacetic acid. The solution was filtered and the filtrate was cooled to permit the phenylacetic acid to recrystallize. Filtration of the mixture yielded about 0.25 gram of phenylacetic acid per gram of potassium benzylpenicillinate. The melting point was checked to assure purity of the acid.

The 0.25 gram of recrystallized phenylacetic acid was then decarboxylated with quinoline and copper chromite according to the method of Huggett (*6*). The carbon dioxide which was evolved was entrained in nitrogen and the mixture was bubbled through excess barium hydroxide. The barium carbonate which formed was isolated under nitrogen. A sample of carbon dioxide for the mass spectrometer was then isolated from the carbonate according to the method of Rittenberg (*8*). The C^{13}/C^{12} ratio was found to be 0.0466.

Preparation of Primary Standard. Potassium benzylpenicillinate was used as the primary standard in the present analytical method. Use was again made of the isopropyl etherate for the preparation of potassium benzylpenicillinate of the desired purity. Commercial benzylpenicillin was converted to the etherate as described by Trenner (*12*). The crude crystals were recrystallized several times according to Trenner's method. The pure etherate was then converted to the potassium salt and recrystallized as before.

Purity of Labeled Standard. The potassium benzylpenicillinate content of the labeled standard was determined by the dilution of 0.25 gram of the material with 1.00 gram of pure potassium benzylpenicillinate, the primary standard. A carbon dioxide sample was isolated from the primary standard, the labeled standard, as well as from a mixture of the two according to the method described previously. The C^{13}/C^{12} ratios of these samples were obtained in the mass spectrometer.

ANALYSIS OF PENICILLIN SAMPLES

Analysis of Broth Samples. In the analysis of broth, 0.25 gram of labeled potassium benzylpenicillinate was added to 3

Table I. Penicillin G in Broth Samples

Sample No.	Labeled Standard Added, G./L. Broth	Bio-assay, μ/Ml.	$C^{13}O_2:C^{12}O_2$ ($C^{12}O_2$ = 100) Electrostat.	$C^{13}O_2:C^{12}O_2$ ($C^{12}O_2$ = 100) Electromag.	Penicillin G as Potassium Benzylpenicillinate, G./L. Broth Electrostat.	Penicillin G as Potassium Benzylpenicillinate, G./L. Broth Electromag.
59	0.083	550	1.91 (1.13)[a]	1.96 (1.16)[a]	0.270	0.265
83	0.090	458	2.02 (1.10)	2.09 (1.16)	0.238	0.235
104	0.093	500	1.96 (1.11)	2.02 (1.16)	0.270	0.270
107	0.087	445	2.01 (1.11)	2.07 (1.16)	0.236	0.232
245	0.083	485	2.08 (1.12)	2.17 (1.17)	0.207	0.197
347	0.083	458	2.19 (1.15)	2.26 (1.20)	0.186	0.184
393	0.083	540	2.16 (1.15)	2.22 (1.20)	0.194	0.195
431	0.083	458	1.91 (1.15)	1.98 (1.19)	0.283	0.276
440 C	0.042	192	2.09 (1.15)	2.16 (1.20)	0.107	0.106
440 D	0.042	208	2.01 (1.15)	2.06 (1.20)	0.122	0.123

[a] Values in parentheses are $C^{13}O_2:C^{12}O_2$ ratios of ordinary CO_2.

liters of the sample. The mixture was filtered. Crystalline potassium benzylpenicillinate was isolated from the filtrate as previously described. The crystalline sample was hydrolyzed to phenylacetic acid. The acid was purified, then decarboxylated to obtain the carbon dioxide sample for assay as described above.

Analysis of Commercial Crystalline Samples. In the analysis of crystalline penicillin, 0.6 gram of a sample was mixed with 0.2 gram of labeled potassium benzylpenicillinate. The mixture was then analyzed according to the procedure described in the experimental section.

CALCULATION OF RESULTS

Purity of the Labeled Standard. The procedure for the isolation of carbon dioxide samples from the primary standard, the labeled standard, as well as from a mixture of the two is described above. The weight of potassium benzylpenicillinate in 0.2500 gram of labeled standard was determined by solving the following equation:

$$X = \frac{A(D - C)(B + 1)}{(B - D)(C + 1)}$$

where X is the weight of potassium penicillin G in a known weight of labeled standard, A is weight of primary standard added, B is the C^{13}/C^{12} ratio of the labeled standard, C is the C^{13}/C^{12} ratio of the ordinary penicillin G, and D is the C^{13}/C^{12} ratio of the mixture of the two. It was found that 0.2500 gram of labeled standard contained 0.229 gram of potassium benzylpenicillinate or 91.6% by weight.

Table II. Penicillin G Crystalline Samples

Code No.	$C^{13}O_2:C^{12}O_2$ ($C^{12}O_2$ = 100) Electrostat.	$C^{13}O_2:C^{12}O_2$ ($C^{12}O_2$ = 100) Electromag.	Potassium Benzylpenicillinate, % by Weight Electrostat.	Electromag.	N.E.P.[a]	Ultraviolet
1	2.03 (1.15)	2.08 (1.20)	70	71	67	..
2	1.90 (1.15)	1.97 (1.20)	87	85	79	79
3	1.86 (1.15)	1.92 (1.20)	93	93	...	93
4	1.87 (1.15)	1.93 (1.20)	91	91	87	88
5	1.97 (1.15)	2.03 (1.20)	77	77	...	68
6	1.79 (1.15)	1.85 (1.20)	101	100	100	..
7	1.91 (1.15)	1.97 (1.19)	85	84	...	78

[a] *N*-Ethyl piperidinium.

Potassium Benzylpenicillinate in Broth Samples. The amount of "penicillin G" in broth samples was determined by dilution of a given amount of sample with a given weight of the labeled standard. The dilution of penicillin in broth with the labeled standard and the isolation of potassium benzylpenicillinate therefrom, as well as the preparation of a carbon dioxide sample from the crystalline product, are described above.

The per cent by weight of potassium benzylpenicillinate in the broth samples was calculated by substituting the known values in the following formula:

$$X = \frac{E(B - D)(C + 1)}{(D - C)(B + 1)}$$

where X is the weight of potassium penicillin G in the unknown sample, B is the C^{13}/C^{12} ratio of the labeled standard, C is the C^{13}/C^{12} ratio of ordinary penicillin G, D is the C^{13}/C^{12} ratio of the mixture of the two, and E is the weight of labeled standard added. In one analysis, a broth sample contained 0.270 gram of penicillin as potassium benzylpenicillinate per liter.

The penicillin G contents of ten samples of broth were determined (Table I).

Penicillin G in Crystalline Samples. The penicillin G contents of seven crystalline samples were determined (Table II). Some of these samples were analyzed by the ultraviolet method (*4*) as well as by the *N*-ethylpiperidinium method (*10*). These data are included in the table for comparison.

DISCUSSION

The penicillin G values that were calculated from the electromagnetic readings were about 1% lower than the values calculated from the electostatic readings. In view of the better agreement among the electromagnetic readings on normal carbon dioxide, it appears likely that these values are more accurate.

In the preparation of penicillin for clinical use, it is necessary to separate penicillin G from the other penicillins during the recovery from the broth. The present method of analysis should prove to be of great value in developing a process for the isolation of penicillin G. The analysis of a broth is of special significance in this connection, inasmuch as it reveals the amount of penicillin G per unit volume. It is recognized that the biological assay of the broth can be obtained more readily, but these data cannot be used to calculate the penicillin G content with the same degree of accuracy.

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Nature of the Cobalt-Thiocyanate Reaction

PHILIP W. WEST AND CHARLES G. DE VRIES[1]
Louisiana State University, Baton Rouge, La.

The nature of the color produced by cobalt-thiocyanate complexes in the presence of certain organic solvents has been investigated, to provide a better understanding of this important color system. The complexes $[Co(NCS)]^+$ and $[Co(NCS)_6]^{----}$ are formed in mixed solvents. The latter complex is probably responsible for the formation of the blue color produced in the Vogel test through its selective attraction of organic molecules. The thiocyanate addenda attract the less polar organic compounds, causing what is in effect a dehydration of the complex cobalt ion and giving rise to the familiar blue color of anhydrous cobalt compounds. This study should aid in providing an understanding of the nature of the Vogel reaction, which is of importance in the detection and determination of cobalt.

ALTHOUGH the reaction of cobalt with thiocyanate ions in the presence of mixed solvents has long been used for the detection and determination of cobalt, the cause of the resulting change in color from a pink to a blue solution has remained unproved and a point of much speculation. This test, named the Vogel reaction after its originator (*21*), consists of adding thiocyanate ions to the solution to be analyzed and then adding a suitable organic solvent. The formation of a blue color indicates the presence of cobalt ions. Because of the widespread use of this reaction it is desirable to gain more knowledge of its exact nature, thus enabling a logical approach for possible improvement of its application.

Many modifications of Vogel's original method of testing for cobalt have been made, including the choice of solvents for color developer (*5–7, 11, 20*) and of ions for complexing addenda (*9, 17*). The use of this reaction as applied to quantitative measurements has not been overlooked. Not only has cobalt been determined colorimetrically, but small percentages of water in ethyl alcohol have been determined accurately employing this color system (*3*).

According to Mellor (*18*), the reaction between a concentrated solution of ammonium thiocyanate and a solution of a cobalt(II) salt first forms cobalt(II) thiocyanate. This salt is converted to ammonium cobalt(II) thiocyanate in the presence of excess thiocyanate ions.

Although a number of theories have been postulated to account for the blue color, no definite explanation is available. Hill and Howell (*13*) proposed that the color change in cobaltous solutions is due to the dehydration of a cobaltous-hexaquo complex ion. In strongly acidic medium (concentrated mineral acids) they suggest that the color of the solution is transformed to blue conforming to the reaction,

$$\underset{\text{Red}}{[CoCl_4(H_2O)_2]^{--}} \rightleftharpoons \underset{\text{Blue}}{[CoCl_4]^{--}} + 2H_2O$$

This dehydration theory was not accepted by Bassett and Croucher (*4*). They claim that the reasoning of Hill and Howell, which was based on the comparison of magnesium and cobalt oxides, is unjustified and that cobalt need not have a coordination number of 6. Rossi (*19*) bubbled dry hydrogen chloride through a 0.1 *M* aqueous cobaltous chloride hexahydrate solution. The solution color changed from a pink to a blue. He suggested that the color is caused by a compound formation of the type,

$$HCoCl_3 . nH_2O$$

One possible explanation for the change of color upon dehydration may be shown by the equation (*8*)

$$\underset{\text{Pink}}{2[Co(H_2O)_6]Cl_2} \rightleftharpoons \underset{\text{Blue}}{Co[CoCl_4]} + 12H_2O$$

[1] Present address, Department of Chemistry, University of Oklahoma, Norman, Okla.

Feigl (*10*) has suggested (very significantly) that the blue color is probably due to solvation of complex cobalt-thiocyanates such as $K_2[Co(NCS)_4]$, inasmuch as upon dilution the color returns to pink.

Young and Hall (*25*) pointed out that the capacity of a solvent for preventing decomposition of a complex varies inversely as its dielectric constant and that the complex formed with cobalt and ammonium thiocyanate is extracted from aqueous solutions by organic solvents.

Absorptancy curves have been run on solutions of cobalt thiocyanate in nonaqueous solvents. In a nonaqueous solvent, *L*, the complex $Co(NCS)_2L_2$ is formed (*16*). The complex $[Co(NCS)]^+$ is present in aqueous solutions containing an excess of cobalt, while an excess of thiocyanate produces $[Co(NCS)_4]^{--}$ according to Kiss and Csokan (*15*).

APPARATUS AND REAGENTS

In the present investigations a Beckman Model DU spectrophotometer was used for all absorptiometric work. The absorption cells were of Corex with a 1.00-cm. path length.

Analytical reagent grade salts of cobalt nitrate and potassium thiocyanate were used in the preparation of standard solutions. The cobalt solutions were standardized by the method of electrolytic deposition, and the strengths of the thiocyanate solutions were found by the Volhard titration using standard solutions of silver nitrate. Absolute ethyl alcohol was used. Potassium selenocyanate was prepared following the method outlined in "Inorganic Syntheses" (*12*).

EXPERIMENTAL

To determine the complexes present in aqueous solutions and in mixed solvents, both Job's method of continuous variation (*22*) and a method employing spectrophotometric titrations (*23, 24*) were applied.

Job's method of determining ionic species by noting the difference in optical density of a system while varying the mole fraction of components is well known. The coordination number of the central atom is found by applying his calculation formula,

$$N = \frac{x}{1 - x}$$

where N = coordination number, and x = mole fraction of complexing ion present at maximum difference in optical density.

The second method, that of spectrophotometric titration, also gives indication as to the nature of complexes formed in solution. A number of solutions are prepared containing the same concentration of the central atom in the coordination sphere. Using these, a series of solutions is made by adding the complexer in amounts ranging from 0 to 15 times the concentration of the central atom present. Optical density measurements are then made at a distinguishing wave length and the coordination combinations are determined from breaks in the slope of the curve of opti-

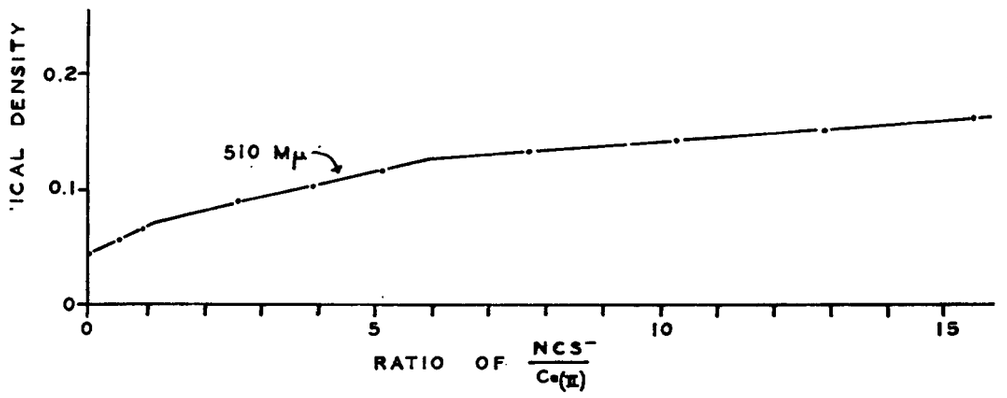

Figure 1. Spectrophotometric Titration
Water solution, 0.00885 *M* Co(II)

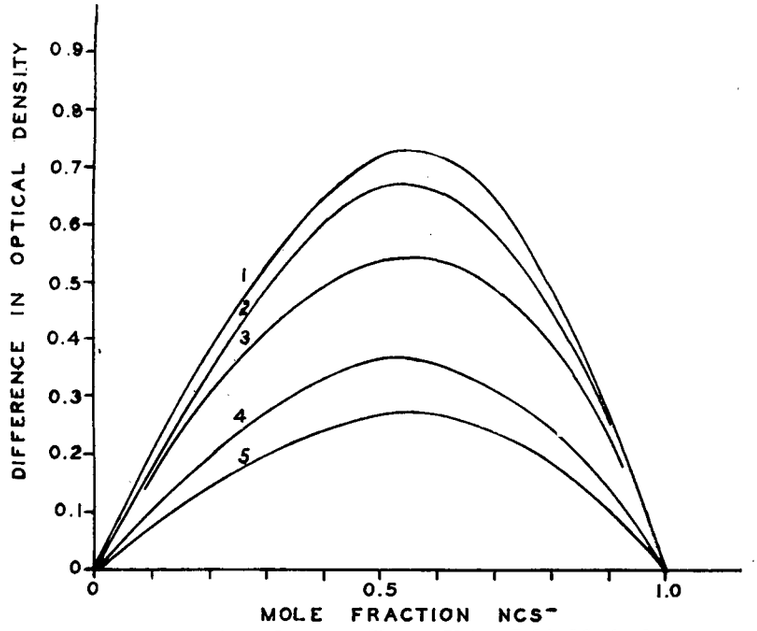

Figure 2. Complexation Shown Using Job's Method

0.200 *M* water solutions of Co(II), NCS^-
1. At 520 mμ
2. At 500 mμ
3. At 480 mμ
4. At 460 mμ
5. At 560 mμ
Slit width at 0.12 mm.

cal density versus ratio of complexer concentration to concentration of central atom.

RESULTS

The complexes of cobalt-thiocyanate formed in aqueous media were found to consist of two species—$[Co(NCS)]^+$ and $[Co(NCS)_6]^{----}$—with the number of possible water molecules in the coordination spheres undetermined. Spectrophotometric titration results, shown in Figure 1, dictate this conclusion. Although the breaks in the curve in Figure 1 are not great, repeated experiments have given the same results. Each gave definite breaks at ratios of 1 to 1 and 1 to 6. (Precision is indicated by the size of the points.) Further proof was obtained by continuous variation studies: A complex of the type $[Co(NCS)]^+$ is clearly indicated, as shown by Figure 2. Moreover, the curve is tilted slightly to the right, thereby indicating the existence of another complex, one richer in thiocyanate.

The curve variation procedure of Job was studied at wave lengths of both 520 and 620 mμ. At the concentrations imposed by the conditions of the experiment it was impossible to perform a spectrometric titration at 620 mμ in aqueous media. Spectral absorptancy curves of optical density versus wave lengths for cobalt-thiocyanate-water solutions were found to have only one peak, at 510 mμ, with no sign of absorption in the blue region of the spectrum.

Results of the investigations of cobalt and thiocyanate ions in mixed media (water–ethyl alcohol) are shown in Figures 3 and 4. A spectrophotometric titration and continuous variation studies, using a wave length of 510 mμ, gave the same results as those obtained with aqueous solutions. However, when these same observations were made using a wave length of 620 mμ, a new species was indicated, for both methods gave irrefutable evidence of the formation of a new type of $[Co(NCS)_6]^{----}$ ion. That the organic solvent is not involved stoichiometrically was proved by the application of both methods, keeping the ratio of cobalt-thiocyanate constant and varying the amount of solvent. Ethyl alcohol at more than forty times the molar concentration of the cobalt was required before the blue color was produced.

Because the organic solvent neither is present in the coordination sphere nor enters into the reaction stoichiometrically, investigations were made as to the effect of change in dielectric constant on optical density in the blue region of the spectrum. A study of a series of alcohols used as developers for the Vogel reaction showed that the blue color increased as the dielectric constant of the developer decreased. Sufficient data could not be found in the literature to correlate the change in optical density with other physical constants of the developers.

All solutions used in the dielectric constant studies had the same concentration of cobalt and thiocyanate ions present and the molarity of the alcohol was kept constant. To 10-ml. volumetric flasks 1 ml. of 0.02 *M* cobalt nitrate and 5 ml. of 1 *M* potassium thiocyanate were added. Enough developer was added to yield a molarity of 4.28 *M* and the solutions were made to volume with distilled water. Optical density measurements were taken at 620 mμ. The results obtained are shown in Table I.

Absorptancy curves of the cobalt-selenocyanate-water system were made and a peak was found to be present at 510 mμ. In mixed media a spectral absorptancy peak at 620 mμ was observed.

Table I. Optical Density

Solution	Developer	Dielectric Constant	Optical Density
1	Methanol	32.6 (*1*)	0.015
2	Ethyl alcohol	25.0 (*1*)	0.090
3	*n*-Propyl alcohol	20.8 (*1*)	0.503
4	Isopropyl alcohol	15.7 (*14*)	0.785
5	*sec*-Butyl alcohol	15.5 (*14*)	0.920
6	*tert*-Butyl alcohol	3.76 (*1*)	1.16

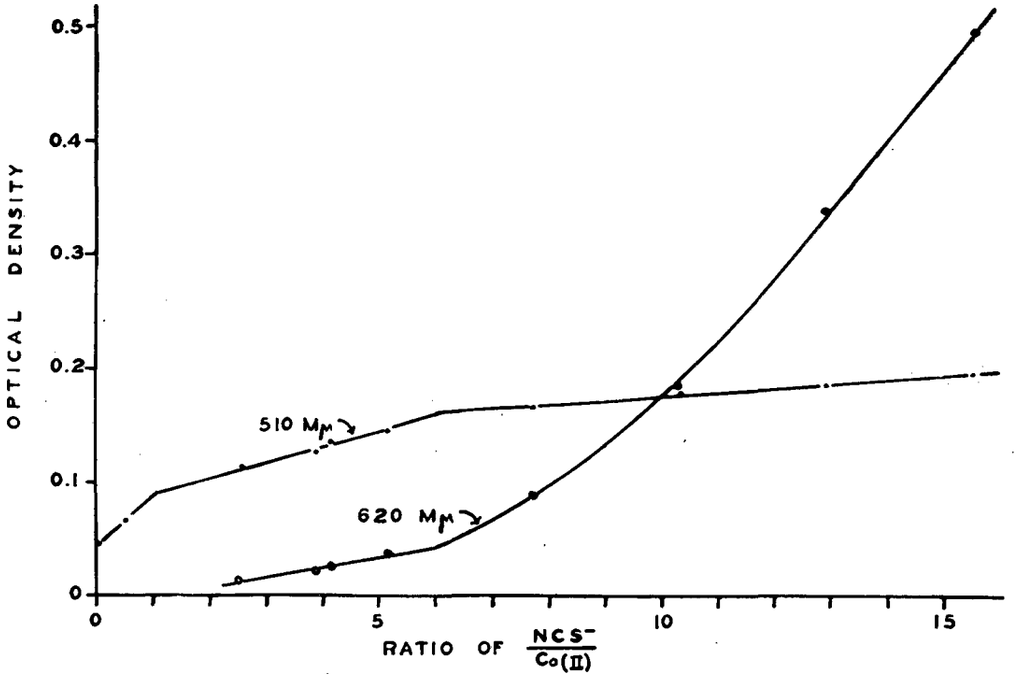

Figure 3. Spectrophotometric Titration

Water-alcohol solutions, 0.00885 *M* Co(II)

DISCUSSION

It has been shown that the complexes, $[Co(NCS)]^{+}$ and $[Co(NCS)_6]^{----}$, exist in both aqueous and mixed solvent systems. In mixed solvents a blue color forms due, it is believed, to the selective attraction of nonaqueous molecules by the thiocyanate of the $[Co(NCS)_6]^{----}$ complex. This preferential attraction of the nonaqueous molecules causes, in effect, a dehydration of the cobalt complex and gives rise to the formation of the familiar blue color of anhydrous cobalt salts.

A considerable amount of evidence, other than that presented above, can be cited in support of the theory. Local investigations have shown that a majority of metal thiocyanates can be extracted from aqueous solutions by means of nonpolar organic solvents. Such extractions have been utilized in a number of ways in the isolation of thiocyanate complexes of such metals as iron, uranium, and molybdenum. Their importance to the present discussion lies in the emphasis they add to the solubility phenomena attributable to the thiocyanate group effect.

Because the color-developing solvents used in the Vogel reaction are miscible with water, no phase separation occurs in this case. The attraction between the thiocyanates and the organic solvent is still in effect, however, and the coordinated thiocyanate consequently attracts the organic molecules to provide an anhydrous atmosphere around the complex. The observation of Young and Hall (*25*) that the blue complex is soluble in ether lends support to the dehydration theory. Studies of dielectric effects also support this view.

The evidence submitted here indicates that the dielectric constant of the developing agent of a chemical series is related to its strength as a color-producing agent in the Vogel test. In the alcohol series which was studied this strength is roughly inversely proportional to its dielectric constant. The observations indicated that other physical factors of a solvent also affect its value as a developer. Although the difference in dielectric constant between isopropyl alcohol and *sec*-butyl alcohol is 0.2, the difference in optical density is proportionally much higher in comparison with the other members of the series. Also, when considering acetone [ϵ = 19.5 (*1*); optical density = 1.10] and dioxane [ϵ = 2.1 (*2*); optical density = 0.885] not in the series, there seems to be no relation between their ability as developers and their dielectric constants.

tert-Butyl alcohol is as effective as acetone as a developer of the blue color. Because the vapor pressure of *tert*-butyl alcohol is so much lower than that of acetone, it seems desirable to investigate it for use in quantitative tests.

Dielectric constant does play a role in the formation of the colored complex, but these data suggest that perhaps other factors, including molecular size, also influence a solvent's ability to produce the Vogel blue.

That this blue color may be associated with the configurations of the electrons in the central atom and not directly with the

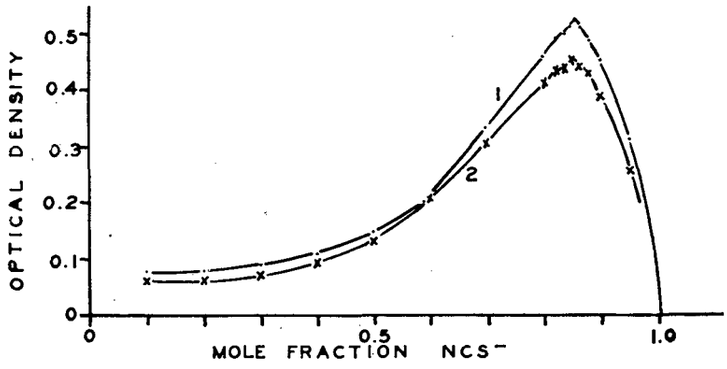

Figure 4. Complexation Shown Using Job's Method

Water-alcohol solutions
Slit width at 0.04 mm.
Co(II) NCS^{-}
1. At 620 mμ
2. At 600 mμ

addenda in the coordination sphere is indicated by the absorption peak at 620 mμ in the spectral absorptancy curves for both the thiocyanate and selenocyanate mixed solvent solutions. Other addenda such as thiosulfate and cyanate also give blue colors with cobaltous ions in mixed solvents. Finally, a wide variety of mixed solvents can be used to develop the blue color, tending to give credence to the dehydration theory.

ACKNOWLEDGMENT

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Titrimetry in Glacial Acetic Acid

Scope of Method

PETER C. MARKUNAS AND JOHN A. RIDDICK, *Commercial Solvents Corp., Terre Haute, Ind.*

This work was undertaken to investigate the possible applications and limitations of titrations in glacial acetic acid. Over 400 compounds have been tested. The analyses of representative groups of compounds that may be titrated in glacial acetic acid are reported. The compounds include salts of strong bases and weak acids, salts of weak bases and weak acids, and organic bases. This study should provide a simple and rapid method for the analysis of carboxylic acid salts of many bases and for a large number of organic bases whose ionization constants (in water) are 10^{-12} or greater; it should, therefore, replace the lengthy and frequently tedious procedures now used for the analysis of these compounds.

TITRIMETRY in nonaqueous solvents is a relatively unexplored field that offers analytical possibilities comparable to those of aqueous titrimetry. However, the published information on this subject does not suggest the widespread applicability nor the distinct advantages of this means of analysis. It is the purpose of this preliminary paper to demonstrate the usefulness of glacial acetic acid as a solvent and perchloric acid as titrant for the direct analysis of weak bases and carboxylic acid salts, and to indicate the general scope of the proposed procedure.

The method used in this study is based upon the observation of Conant and Hall (*2*) that many substances which show little or no basic properties in water behave as strong bases in acetic acid, and upon the studies of Hall and Werner (*4*), who demonstrated the basic character of carboxylic acid salts in acetic acid, and showed that perchloric acid is the strongest and most suitable titrant. Kolthoff and Willman (*5*) confirmed the basic character of several inorganic acetates in acetic acid and measured their basic strength in this solvent. Blumrich and Bandel (*1*) reported that the alkali, alkaline earth, and ammonium salts of carboxylic acid salts could be titrated in acetic acid, but they gave no data to support their statement. No systematic study has been made to apply these observations to the analysis of either weak bases or to the salts of carboxylic acids. The authors are making this study.

The following analytical applications have been made of the known acid-base relationships in acetic acid: titration of aniline, pyridine, and other weak organic bases (*1*), quantitative titration of amino acids (*6*), determination of tertiary aliphatic amines in the presence of primary and secondary amines (*1*, *9*), determination of basic nitrogen compounds in refined hydrocarbons (*10*) and in hydrocarbon oils and coal hydrogenation oils (*11*); and determination of amino sulfonamides (*8*).

This paper presents a summary of the survey analysis of several hundred compounds. This information will be of use to many analysts even though the method presented herein was limited to relatively dry, pure materials and absolute accuracy and precision remain to be established.

REAGENTS, SOLUTIONS, AND APPARATUS

Acetic acid, glacial, A.C.S. grade.
Acetic anhydride, A.C.S. grade.
Perchloric acid, 70 to 72%, A.C.S. grade.
Potassium acid phthalate, primary standard grade.
Perchloric Acid Solution, 0.1 *N*. The standard acid solution is prepared by adding 14.5 grams of 70 to 72% perchloric acid to approximately 900 ml. of glacial acetic acid in a 1-liter volumetric flask, slowly adding, while constantly swirling the flask, sufficient acetic anhydride to react with the water introduced with the perchloric acid, and diluting to the mark with acetic acid. This solution is allowed to stand 24 hours and is stand-

Table I. Analysis of Carboxylic Acid Salts

Chemicals[a]	Determined Purity, % Indicator method	Potentiometric method
Ammonium acetate		98.29
Ammonium benzoate	98.09	98.04
Ammonium citrate		99.64
Ammonium salicylate	99.55	99.69
Sodium acetate	99.02	99.14
Sodium formate		99.36
Sodium citrate		99.60
Sodium salicylate	99.32	99.50
Sodium benzoate	99.22	99.46
Potassium acetate	98.24	98.12
Potassium gluconate		99.40
Calcium acetate	....	99.17
Calcium formate		99.19
Barium acetate	....	99.23
Magnesium acetate	....	101.7
Lithium benzoate	....	99.92
Lithium salicylate		98.83
Guanidine acetate[b]	99.62	99.62
8-Hydroxyquinoline benzoate	....	98.81
2-Hydroxyethyltrimethylammonium dihydrogen citrate	99.97	100.01

[a] Purchased C.P. chemicals except guanidine acetate.
[b] Eastman Kodak white label chemicals.

ardized with potassium acid phthalate either potentiometrically or to the crystal violet end point as described in the next section.

Crystal violet indicator, 1 gram of crystal violet dissolved in 100 ml. of acetic acid

Precison-shell titrometer (*7*) with calomel and glass electrodes was used for potentiometric titrations.

ANALYTICAL PROCEDURES

Potentiometric Method. A sample containing about 3.5 milliequivalents of salt is weighed into a 250-ml. tall-form beaker, without spout, and dissolved in 30 ml. of acetic acid, and the mixture is titrated potentiometrically with 0.1 *N* perchloric acid in acetic acid. The titration is conducted and the equivalent point determined in the same manner as for potentiometric titration in aqueous solutions.

Indicator Method. A sample the same weight as required for the potentiometric method is weighed into a 250-ml. Erlenmeyer flask, dissolved in 30 ml. of acetic acid, warmed if necessary to effect solution, and cooled. One drop of crystal violet indicator is added and the solution is titrated with 0.1 *N* perchloric acid in acetic acid to a blue-green color.

EXPERIMENTAL

The potentiometric and indicator methods were used in a comprehensive survey to determine the probable applicability of nonaqueous titrimetry to different types of compounds. Materials of the highest purity obtainable were used without further purification. This permitted scanning a large number of compounds in a reasonable length of time. The results of analysis of certain salts of some carboxylic acids were selected to illustrate some phases of the applicability of the method. The analysis of salts of some carboxylic acids is given in Table I. A few typical titration curves of these salts are shown in Figure 1.

In order to correlate results with those of Hall (*3*), some organic bases were analyzed by the methods used for the determination of carboxylic acid salts. The results are given in Table II and the potentiometric titration curves for a few of these compounds are shown in Figure 2. These data confirm those of Hall and illustrate the general usefulness of the proposed method for the estimation of weakly basic amines.

The analytical data presented in Tables I and II are the average of at least two determinations which agreed at least to 5 parts per thousand.

DISCUSSION

Those carboxylic acid salts and organic bases which show a sharp break in the titration curve may be estimated by the more rapid indicator method with the accuracy and precision obtainable by the potentiometric method. It is recommended, however, that use of the indicator method for any particular compound should first be tested by trying the potentiometric and indicator titrations simultaneously on the compound to be analyzed.

Although the precision and accuracy of the method are still being investigated and will be presented in a subsequent paper, the present results indicate that this method is as accurate and precise as aqueous acidimetric and alkalimetric methods. The reproducibility for compounds that act as strong bases in glacial acetic acid for routine type determinations is about 0.2%.

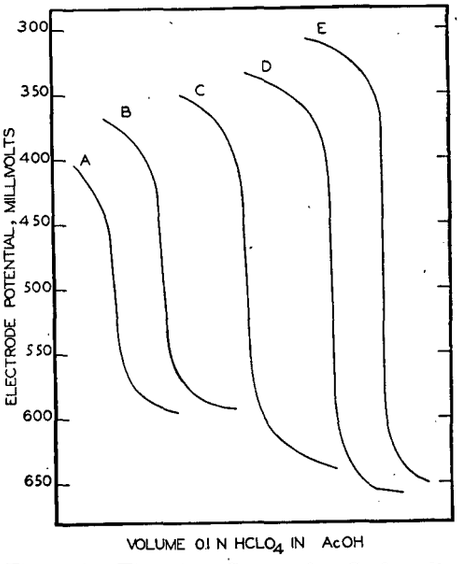

Figure 1. Titration Curves for Carboxylic Acid Salts

A. **Lithium benzoate**
B. **Sodium acetate**
C. **2-Hydroxyethyltrimethylammonium dihydrogen citrate**
D. **Ammonium acetate**
E. **Potassium acetate**

In one study, the effect of water upon the titration of 2-hydroxyethyltrimethylammonium dihydrogen citrate in glacial acetic acid was extensively studied. Large amounts of water gave high results but amounts up to approximately 1.5% of the original solvent (30 ml.) did not affect the accuracy of the de-

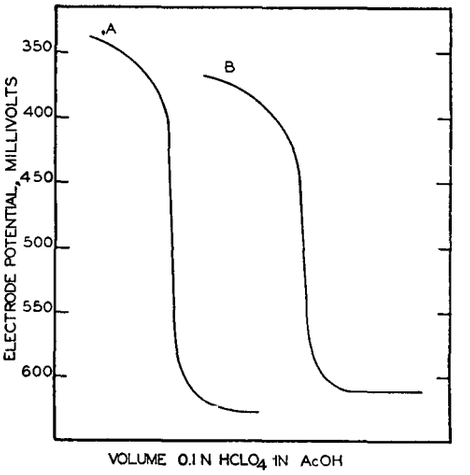

Figure 2. Titration Curves for Organic Bases

A. **Diethylaniline**
B. **2-Nonyl-4,4-bis(hydroxymethyl)-2-oxazoline**

Table II. Analysis of Organic Bases by Direct Titration

Chemicals	Determined Purity, % Indicator method	Potentiometric method
Diethylaniline[a]	99.17	99.36
8-Hydroxyquinoline[a]	99.45	99.78
Diphenylguanidine[a]	....	99.91
Aniline[b]	99.41	....
n-Butylaniline[a]	99.32	
Tris(hydroxymethyl)aminomethane[c]		99.94
Benzocaine[b]	....	99.20
2-Nonyl-4,4-bis(hydroxymethyl)-2-oxazoline[d]	99.85	100.00

[a] Eastman Kodak white label chemicals.
[b] Purchased C.P. chemicals.
[c] Purified by recrystallization from methanol, m.p. 171.1° C.
[d] Purified by recrystallization from benzene, m.p. 91.2 to 91.6° C.

termination. In fact, it is possible to analyze aqueous solutions of this compound by a slight modification of the method given herein. (A report on this application is being prepared.)

A study of the results of this survey shows that the method is applicable to the analysis of salts of strong bases and weak acids, salts of weak bases and weak acids, and weak organic bases. The major limitation of the method is the insolubility of some of the compounds in acetic acid. The authors have found that some compounds they predicted could be analyzed do not react stoichiometrically; the reason for this is not yet apparent.

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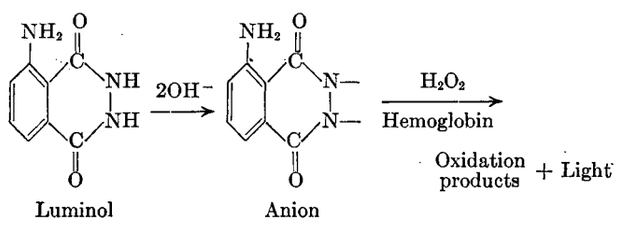
Luminol as a Chemiluminescent Indicator

In Acid-Base Titrations with a Dark-Chamber Titrimeter

FREDERIC KENNY AND R. B. KURTZ, *Hunter College, New York, N. Y.*

Luminol indicator is a new type of acid-base indicator which emits light at the end point. It was developed to make possible acid-base titrations in solutions having sufficient color to prevent the use of ordinary indicators, and at the same time avoid the use of a potentiometric titration. The indicator, consisting of luminol, hemoglobin, and hydrogen peroxide, emitted light at a pH very close to the stoichiometric point in the titration of 0.1 *M* hydrochloric acid with 0.1 *M* sodium hydroxide. The presence of a highly colored component such as gentian violet did not interfere with the end point. No indicator error was observed in the titration of either colorless or colored solutions. This new indicator should have wide application in titrating a variety of colored solutions.

AFTER careful consideration of various chemiluminescent materials that might be used as indicators in acid-base titrations, it was decided to use luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), hydrogen peroxide, and hemoglobin, because this combination is sensitive to small variations in pH. In each titration approximately 40 mg. of luminol, 6 ml. of 3% hydrogen peroxide, and 30 mg. of hemoglobin were used.



Luminol Anion

This indicator emits light above a given pH, as contrasted with ordinary indicators, which absorb light differently above a given pH. Ordinary indicators function alone, whereas luminol, at room temperature and in aqueous solution, functions only when associated with hydrogen peroxide and a catalyst. Ordinary indicators are not destroyed during the color change, whereas luminol is consumed at the pH at which light and oxidation occur. Thus the indicator reaction is irreversible. It lends itself to titration of acid with base. If, however, a base is to be titrated with acid, a measured excess of acid must be added, followed by back-titration with base. The behavior of the indicator may be summarized by the formula in column 1.

It is necessary for the hydroxyl ions to remove protons from the luminol to form the anion before the reaction producing light can proceed (*20*, *21*). The oxidation processes which emit light do not start until the solution pH is increased to a point close to the neutral point. This critical pH value is the basis for the accompanying method of acidimetry. The maximum chemiluminescence intensity as a function of the molarity of the sodium hydroxide present (*21*, *22*) shows a sharp maximum at 0.01 to 0.05 *M*, corresponding to pH values between 12 and 12.7. A perceptible light appears, however, when sufficient sodium hydroxide has been added to give a pH in the neighborhood of 7.

Table I. Volume of Sodium Hydroxide Solution Equivalent to 30.00 Ml. of Hydrochloric Acid

Phenolphthalein as indicator and luminol as a chemiluminescent indicator)

	Phenolphthalein		Luminol	
	Sodium hydroxide, ml.	Deviation from mean, ml.	Sodium hydroxide, ml.	Deviation from mean, ml.
	31.80	0.02	31.82	0.00
	31.82	0.00	31.75	0.07
	31.88	0.06	31.78	0.04
	31.80	0.02	31.90	0.08
	31.86	0.04	31.80	0.02
	31.81	0.01	31.80	0.02
	31.80	0.02	31.90	0.08
			31.80	0.02
Mean	31.82	0.02	31.82	0.04

Table II. Volume of Sodium Hydroxide Solution Equivalent to 30.00 Ml. of Hydrochloric Acid

(Luminol indicator in presence of 0.003% gentian violet)

Titration	Sodium Hydroxide, Ml.	Deviation from Mean, Ml.
1	31.90	0.08
2	31.80	0.02
3	31.89	0.07
4	31.89	0.07
5	31.75	0.07
6	31.89	0.07
7	31.70	0.12
8	31.77	0.05
9	31.68	0.14
10	31.80	0.02
11	31.80	0.02
12	31.90	0.08
13	31.75	0.07
14	31.90	0.08
Mean	31.82	0.07

In the present research this light has been used to mark the end point of the titration of acid with base.

The catalyst used must be stable in both acid and alkaline solution. Soluble complex salts of transition metals such as cobalt and especially iron in the form of hemin, hemoglobin, or ferricyanide are examples. Substances like permanganate and hypochlorite, while bringing about the evolution of light, are rapidly destroyed, whereas hemoglobin can function for a much longer time (*21*). Theories for the behavior of hemoglobin have been formulated by a variety of investigators, some of whom regard it as a peroxidase reaction (*1, 5, 11, 14; 17*, pp. 268, 295; *18*, pp. 249, 252, 268, 274; *21*).

The function of hydrogen peroxide is to bring about the oxidation of the anion derived from the luminol. The manner in which it does this has been the subject of much speculation (*2, 5, 21*). According to recent ideas, the hydrogen peroxide may furnish free radicals to the solution (*3–5, 9, 10, 16–19*), which when reacting with the anion of the luminol in the presence of hemoglobin, produce a series of transient intermediate free radicals (*2, 5–7, 12, 21*) and the evolution of light from the final diradical (*15*). These changes may involve the transient formation and immediate dissociation of transannular peroxides (*8*).

The concentrations of the three components of the indicator may vary over rather wide limits. For luminol the limits include 0.01 to 1%, for hemoglobin 0.001 to 1%, and for hydrogen peroxide 0.03 to 1%. In this research these three components were, respectively, 0.1, 0.1, and 0.3% at the beginning of the titration.

Use and Advantages of Luminol Indicator. There are many situations in which an acid-base titration cannot be carried out with ordinary acid-base indicators, because the solution contains a highly colored component which makes it impossible to observe the color change of the indicator. Such situations have been dealt with heretofore either by the use of an instrumental method or, if feasible, by the prior removal of the colored component. The use of luminol makes it possible to carry out the titration in the presence of the colored component and without the use of electrical instruments.

EXPERIMENTAL

Approximately 0.1 *M* solutions of hydrochloric acid and sodium hydroxide were prepared. Seven 30.00-ml. portions of the hydrochloric acid were titrated with the sodium hydroxide, using phenolphthalein as the indicator. The end point was taken as the faintest observable pink color.

Eight 30.00-ml. portions of the hydrochloric acid were then titrated with the sodium hydroxide, using the luminol indicator, and carrying out the titrations in the dark-chamber Titrimeter (*13*).

The results of both sets of titrations are shown in Table I.

When phenolphthalein was used as the indicator, the average deviation of a single observation obtained was 0.63 part per 1000. The luminol, however, yielded a precision of 1.25 parts per 1000.

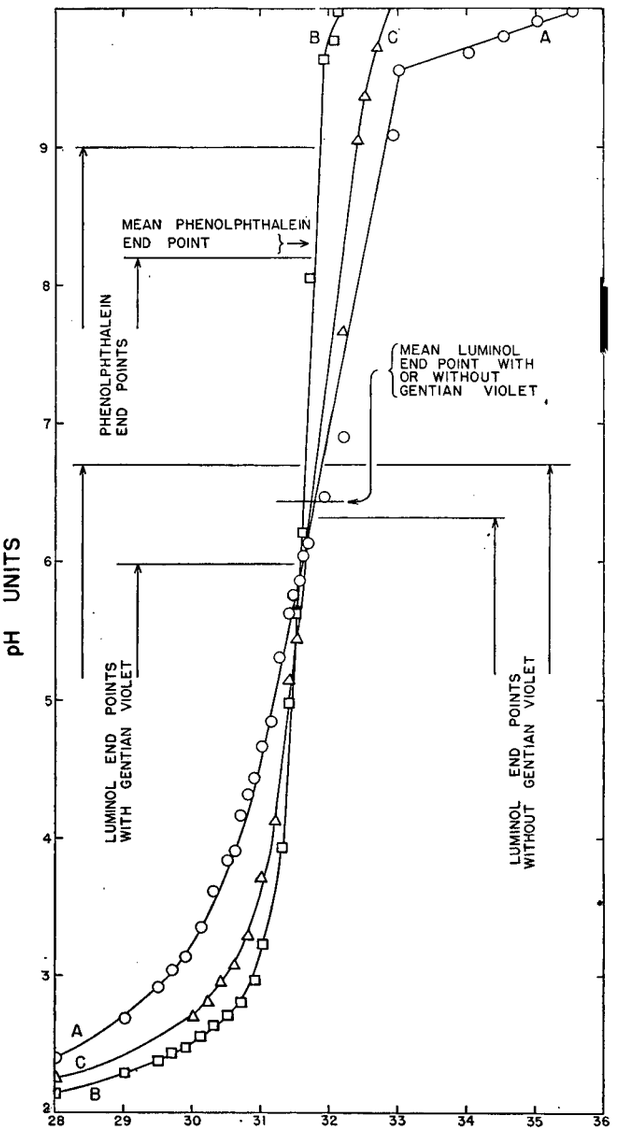


Figure 1. Titration of Hydrochloric Acid with Sodium Hydroxide

A. **Using luminol indicator**
B. **Using phenolphthalein indicator**
C. **Using luminol indicator with hydrogen peroxide omitted**

Both indicators yielded a mean of 31.82 ml. of sodium hydroxide equivalent to 30.00 ml. of hydrochloric acid. Thus the indicator error was negligible.

In order to study the behavior of the luminol indicator in the presence of a highly colored component, 14 titrations of the hydrochloric acid with the sodium hydroxide were carried out, using the luminol indicator in the presence of gentian violet. The concentration of gentian violet used—0.003%—gave the solution an intense color which made it utterly impossible to observe the end point of any of the ordinary acid-base indicators.

The results of these titrations, shown in Table II, yield a mean value for the volume of sodium hydroxide required which is the same as that required in both titrations shown in Table I—namely, 31.82 ml. The deviation from the mean, however, is somewhat larger. Its value—0.07 ml.—corresponds to a precision of 2.13 parts per 1000.

In order to study more carefully titrations in which luminol indicator is used, and to account, if possible, for the lower precision obtained with luminol as compared with phenolphthalein, five titrations of hydrochloric acid with sodium hydroxide were carried out potentiometrically in the presence of the luminol indicator. A glass indicator electrode, a saturated calomel reference electrode, and a Leeds and Northrup 7660-A vacuum tube potentiometer were employed. The temperature of the laboratory was 25° ± 2° C.

The average curve obtained is plotted in Figure 1, *A*. The potentiometric titration of the hydrochloric acid with the sodium hydroxide in the absence of luminol indicator is plotted as *B*.

The two curves cross very close to the luminol end point. The more gradual slope of the luminol curve accounts for the lower precision obtained with this indicator. Work is in progress to modify the composition of the luminol indicator, so as to give a curve with a steeper slope, thereby improving the possibility of obtaining a higher degree of precision. If the titrations employing luminol in both Tables I and II are considered, the over-all precision obtained is 1.85 parts per 1000. Indications at the present time are that this can be improved upon.

The more gradual slope of *A* as compared with *B* is to be expected, in view of the fact that each component of the indicator functions as a weak electrolyte. Because both luminol and hemoglobin combine with hydrogen ion, the pH values preceding the stoichiometric point will be higher than otherwise, and because these same components supply protons to hydroxyl ions after the stoichiometric point has been passed, the pH values obtained will be lower than in the absence of these components. This effect is further accentuated by the presence of the weak acid hydrogen peroxide, which supplies protons to hydroxyl ions in the neighborhood of the stoichiometric point.

As an aid to further study of the luminol indicator a potentiometric titration was carried out using luminol indicator from which the hydrogen peroxide had been omitted. The results are plotted as *C* in Figure 1. Part but not all of the buffering action of the indicator appears to be caused by the hydrogen peroxide.

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Polarographic Behavior of Organic Compounds

Analysis of Mixtures of Dichloroacetic and Trichloroacetic Acids

PHILIP J. ELVING AND CHING-SIANG TANG

The Pennsylvania State College, State College, Pa.

ELVING and Tang (*1*) reported that in the pH range of 6.8 to 10.4 and the potential range of 0.4 to −1.9 volts, acetic and monochloroacetic acids give no polarographic wave, while trichloroacetic acid gives two waves and dichloroacetic acid gives one wave; the latter wave is identical in characteristics with the more negative wave of trichloroacetic acid. The diffusion currents of each of the two waves of trichloroacetic acid and of the one wave of dichloroacetic acid, when corrected for the effect of the electrocapillary curve, are identical; the diffusion currents are directly proportional to the concentration of the acids. The waves are due to the successive removal of halogen whereby trichloroacetate is converted to dichloroacetate, which can then be reduced to monochloroacetate.

In the procedure described for analyzing mixtures of dichloroacetic and trichloroacetic acids, the latter is determined from the diffusion current of its first wave, while the dichloroacetic acid can be measured by deducting the adjusted diffusion current of the first wave of the trichloroacetic acid from the total diffusion current of the second wave. The standard series method of calibration can be used.

A means was sought for the determination of trichloroacetic and dichloroacetic acids in the presence of each other and of related compounds. Dichloroacetate and trichloroacetate, present singly or in mixture, can be determined simply and rapidly from the polarogram obtained at pH 8 in buffered solution. The trichloroacetate gives two reduction waves representing the successive removal of halogen to form first dichloroacetate and then monochloroacetate; dichloroacetate gives one wave which is identical in characteristics with the second wave of trichloroacetate. Monochloroacetic acid does not affect the determination unless its molar concentration is greater than five times that of the dichloroacetic acid or seventy-five times that of the trichloroacetic acid. Acetic acid does not interfere. The method of polarographic analysis presented is suitable for the rapid and simple determination of trichloroacetate and dichloroacetate individually and in the presence of each other. Substances polarographically inactive in the potential range covered do not interfere.

EXPERIMENTAL WORK

Reagents and Chemicals. All reagents used were of C.P. quality. Stock standard solutions of trichloroacetic acid (10 millimolar), dichloroacetic acid (10 millimolar), monochloroacetic acid (1.0 *M*), and acetic acid (1.0 *M*) were prepared, and were standardized by titration with a standard sodium hydroxide solution, using phenolphthalein as indicator. Double-strength buffer solution of pH 8.2 was prepared by adding concentrated ammonium hydroxide to a 1.0 *M* solution of ammonium chloride until the desired pH was reached; only a few drops per liter are necessary. When the solution is diluted in use, the electrolyte content is great enough so that the buffer solution can act as the base solution.

Apparatus. A calibrated Fisher Elecdropode was used in most of the work; all measurements were made at 0.2, 0.1, or 0.05 of the galvanometer sensitivity. The deflection of the Elecdropode galvanometer scale was calibrated in microamperes by substituting for the polarographic cell Akra-Ohm resistances ranging from 10,000 to 100,000 ohms. Some current-potential curves were determined with a Sargent Model XXI polarograph. A Beckman Model G pH meter was used for the measurement of pH. All glassware used was borosilicate; all measuring apparatus was calibrated.

Table I. Effect of Concentration of Acetic and Monochloroacetic Acids on Diffusion Currents of Dichloroacetic and Trichloroacetic Acids

Sample Taken				Trichloroacetic Acid				Dichloroacetic Acid	
TCA	DCA	MCA	AA	First Wave		Second Wave[a]			
mM	*mM*	*mM*	*mM*	*μa.*	*mM*	*μa.*	*mM*	*μa.*	*mM*
0.100	..	2.5	..	0.812	0.099	0.814	0.101	..	..
0.100	..	5.0	..	0.814	0.100	[b]	..	..	..
1.01	..	5.00	..	8.12	0.99	8.14	1.00	..	..
1.01	..	7.50	..	8.14	0.99	[b]	..	..	..
1.01	..	50.0	..	8.14	0.99	[b]	..	..	..
1.01	..	75.0	..	[b]		[b]			
2.02	..	15.0	..	16.30	1.98	16.31	2.00	..	..
2.02	..	25.0	..	16.30	1.98	[b]		..	..
1.01	..	..	100	8.12	0.99	8.15	1.00	..	..
..	1.02	5.00	..	..	..	..	..	8.23	1.00
..	1.02	7.50	..	..	..	..	..	[b]	[b]
..	1.02	..	100	..	..	..	..	8.22	1.00
1.01	1.02	15.0	..	8.14	0.99	..	[c]	8.20	1.00

TCA. Trichloroacetic acid. MCA. Monochloroacetic acid.
DCA. Dichloroacetic acid. AA. Acetic Acid.

[a] Diffusion current for second wave corrected for effect of electrocapillary curve.
[b] Beginning of wave due to monochloroacetic acid merged with waves due to polychloroacetic acids.
[c] Diffusion current and concentration for second wave of trichloroacetic acid are not reported, because in a mixture they are obtained by calculation from data for first wave.

Basis for Procedure. The procedure followed in the polarographic study of trichloroacetic and dichloroacetic acids (*1*) was used as the basis for the analytical method. The half-wave potentials at 25° *vs.* the saturated calomel electrode are −1.57 volts for dichloroacetate, and −0.84 and −1.57 volts for trichloroacetate; these are constant over the pH range of 6.8 to 10.4. Optimum waves are obtained in buffer solution of pH 8, which is 0.5 *M* in electrolyte. Dissolved oxygen need not be removed; the residual current obtained with the base solution is subtracted from the limiting current obtained for the sample solution. The height of the second wave is corrected for the effect of the electrocapillary curve in the usual manner (*2*), the capillary constants having been measured in the buffer base solution used.

Although the polarographic measurements can be made at any temperature, some type of constant temperature arrangement is desirable in order to ensure that the calibration and sample measurements are made at the same temperature—e.g., 25° C.

Table II. Calibration Data for Dichloroacetic and Trichloroacetic Acids

Sample Taken		First Wave	Second Wave[a]
	mM.	*μa.*	*μa.*
TCA	0.098	0.80	0.79
	0.492	4.04	4.00
	0.986	8.11	8.04
	1.97	16.21	16.05
DCA	0.100	...	0.81
	0.500	...	4.09
	1.00	...	8.20
	2.00	...	16.35

[a] Diffusion current for second wave corrected for effect of electrocapillary curve.

Calibration Procedure. In the experimental work or in calibration runs known volumes of the stock solutions of the acids are pipetted into a 100-ml. calibrated volumetric flask, 50 ml. of the 1.0 *M* ammonium chloride–ammonium hydroxide base solution are added, and the contents are diluted to the mark. In this way, the resulting solution has known concentrations of acids and of base solution. The variation in pH of the mixture from the original buffer solution is negligible. The electrolysis is performed as subsequently indicated.

ANALYTICAL PROCEDURE

Measure out a sample containing between 15 and 35 mg. of trichloroacetic acid, and between 1.5 and 25 mg. of dichloroacetic acid; the amount of monochloroacetic acid should not exceed 50 mg. if dichloroacetic acid is present and 750 mg. if dichloroacetic acid is absent. Transfer the sample to a 100-ml. calibrated volumetric flask; add 50 ml. of the 1.0 *M* ammonium chloride–ammonium hydroxide base solution; carefully adjust to pH 8.2 by the dropwise addition of concentrated ammonium hydroxide; and dilute the contents to the mark. Rinse the cell and electrode several times with the solution to be analyzed. Electrolyze the solution, using a quiet mercury pool or a saturated calomel as the reference anode electrode, over the potential range of −0.4 to −1.9 volts *vs.* the saturated calomel electrode. If the electrocapillary curve for the base solution is not known, note t, the drop time, at potentials of −1.3 and −1.8 volts *vs.* S.C.E. Run a similar curve on the base solution and correct the sample curve for the latter curve.

Using the intercept method, determine the diffusion current for each of the two waves. The diffusion current for the first wave is used to calculate the amount of trichloroacetic acid present; the diffusion current used to calculate the dichloroacetic acid present can be determined from the following relation:

$$_2i_d = {}_ci_d - {}_1i_d \frac{{}_2t^{1/6}}{{}_1t^{1/6}}$$

Table III. Analysis of Mixtures of Dichloroacetic and Trichloroacetic Acids

Sample Taken		First Wave		Second Wave[a]				Error			
TCA	DCA	$_1id$	TCA	$_1id$	0.94 $_1id$	$_2id$	DCA	TCA	DCA	TCA	DCA
mM	*mM*	*μa.*	*mM*	*μa.*	*μa.*	*μa.*	*mM*	*mM*	*mM*	%	%
0.098	0.100	0.76	0.093	1.57	0.71	0.86	0.106	−0.005	+0.006	−5.0	+6.0
0.098	0.500	[b]		5.0	(4.9)						
0.098	1.00	[b]		8.9	(9.0)						
0.098	2.00	[b]		17.2	(17.1)						
0.492	0.100	4.00	0.487	4.56	3.76	0.80	0.099	−0.005	−0.001	−1.0	−1.0
0.492	0.500	4.00	0.487	7.98	3.76	4.22	0.516	−0.005	+0.016	−1.0	+3.2
0.492	1.00	3.95	0.481	11.98	3.71	8.27	1.01	−0.011	+0.01	−2.2	+1.0
0.492	2.00	[b]		21.1	(20.4)						
0.986	0.100	8.11	0.986	8.44	7.62	0.82	0.101	0.00	+0.001	0.0	+1.0
0.986	0.500	8.08	0.982	11.73	7.60	4.13	0.505	−0.004	+0.005	−0.4	+1.0
0.986	1.00	8.07	0.981	15.8	7.59	8.21	1.00	−0.005	+0.00	−0.5	0.0
0.986	2.00	7.95	0.967	23.9	7.47	16.43	2.01	−0.019	+0.01	−1.9	+0.5
1.97	0.100	16.2	1.97	16.0	15.2	0.80	0.099	−0.00	−0.001	0.0	−1.0
1.97	0.500	16.1	1.96	19.2[c]	15.1	4.10	0.501	−0.01	+0.001	−0.5	+0.2
1.97	1.00	16.1	1.96	23.3[c]	15.1	8.20	1.00	−0.01	0.00	−0.5	0.0
1.97	2.00	16.1	1.96	31.5[c]	15.1	16.4	2.00	−0.01	0.00	−0.5	0.0

[a] Ratio of sixth roots of drop times was 0.94 for capillary used in making these measurements.
[b] Waves merged, but total wave is compared to that expected from calibration runs.
[c] Galvanometer reading on limiting current plateau of second wave is not very steady, but diffusion current can still be measured.

where $_1i_d$ and $_ti_d$ are the diffusion currents measured after the first and second wave increments at −1.3 and −1.8 volts, respectively, and $_1t$ and $_2t$ are the drop times on the limiting current portions of the first and second waves, respectively—i.e., at −1.3 and −1.8 volts.

The weights or percentages of the two acids present are calculated as with the standard series technique of measurement.

DATA

The effect of acetic and monochloroacetic acids on the diffusion currents of dichloroacetic and trichloroacetic acids is given in Table I.

A typical set of calibration data (concentration *vs.* diffusion current) for the two acids is given in Table II; linear relations are obtained. The diffusion current constants, $i_d/Cm^{2/3}\,t^{1/6}$, at 25° are 4.63 for dichloroacetic acid, and 4.64 and 4.63 for the first and second waves, respectively, of trichloroacetic acid. The effect of one acid on the other in mixtures is summarized in Table III; blank spaces indicate sufficient merging of the two waves to make separate measurement of the waves inaccurate.

With low concentration of the acids, no maxima were found; slight maxima were obtained in most cases with concentration exceeding 2 millimolar, but were never found to interfere with measurement of diffusion current.

DISCUSSION

Although reference throughout this paper has been to the acids, at pH 8.2 the concentrations of the undissociated forms of the acids are negligible and the behavior observed is that of the anions.

The data obtained in these studies indicate that in samples (1) where the concentration of trichloroacetic acid is between 1 and 2 millimolar and the dichloroacetic acid is between 0.1 and 2 millimolar, the percentage error is 0 to 2% for each acid; (2) where the trichloroacetic acid is 0.1 to 0.5 millimolar and the dichloroacetic acid exceeds 2 millimolar, the first wave of trichloroacetic is not clear; and (3) where the trichloroacetic acid is less than 0.1 millimolar with any concentration of dichloroacetic acid, the plateau of the first wave becomes less flat and coincides with the second wave.

If the amount of dichloroacetic acid exceeds the amount of trichloroacetic acid by a fourfold factor, it would be necessary to add known amounts of trichloroacetic acid to bring up the concentration in order to obtain optimum results. The real limitation of the technique described is that for 1 millimolar concentrations of the polychlorinated acids the molar concentration of the monochloroacetic acid should not exceed five times that of the dichloroacetic acid and seventy-five times that of the trichloroacetic acid; for 0.1 millimolar concentrations of the polychlorinated acids, the ratios are 2.5 and 75. For 2 millimolar concentrations of the polychlorinated acids the ratios are 12.5 and 75. The presence of more than a fivefold greater concentration of monochloroacetic acid will affect the second wave of trichloroacetic acid or the wave of dichloroacetic acid, because the beginning of the wave due to the reduction of monochloroacetic acid merges with these latter waves. The presence of more than a seventy-five times greater concentration of monochloroacetic acid will affect the first wave of trichloroacetic acid, because the wave due to monochloroacetic acid merges with the first trichloroacetate wave.

With a mixture containing only the three chloroacetic acids, polarographic analysis plus determination of the total acidity by titration can be used to determine the amount of each acid. The difference between the total acidity and the sum of the dichloroacetic and trichloroacetic acids present gives the amount of monochloroacetic acid. The polarographic reduction of other polyhalogenated compounds is being studied.

SUMMARY

Based upon their polarographic reduction in buffer solution of pH 8, dichloroacetate and trichloroacetate can be simply and rapidly determined when present either separately or in mixture. Trichloroacetate gives two waves, the second, more negative one, of which is identical in characteristics with the one wave of dichloroacetate; the half-wave potentials are −0.84 and −1.57 volts *vs.* S.C.E. The diffusion currents of all three waves are identical and are proportional to concentration; the diffusion current constants are 4.64 and 4.63. The analytical results show an accuracy of 2% or better. Excessive amounts of monochloroacetate interfere; acetic acid does not interfere.

ACKNOWLEDGMENT

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Correction

Attention has been called to an error in the article entitled "Principles of Precision Colorimetry. Measuring Maximum Precision Attainable with Commercial Instruments" [ANAL. CHEM., **22**, 1464 (1950)]. On page 1467 the correct range for the absorption-spectrum data on the copper(II) ammonium ion is 560 to 760 mμ.

C. F. HISKEY

Polarographic Determination of Dimethylamine

F. L. ENGLISH

Chambers Works, E. I. du Pont de Nemours & Co., Deepwater, N. J.

A procedure is presented for the determination of dimethylamine in crude liquors containing, in addition to dimethylamine, ammonia, monomethylamine, and trimethylamine. The sample is treated with nitrous acid which destroys ammonia and most of the monomethylamine, converts dimethylamine to the nitrosamine, and leaves trimethylamine substantially unaffected. After adjusting to a pH of 0.8, the nitrosamine is determined polarographically; the error is about ±1% of the amount present.

A POLAROGRAPHIC technique for the estimation of small amounts of dimethylamine has been reported by Smales and Wilson (*5*) which depends upon conversion to the nitroso derivative. Their procedure involves a distillation which is undesirably long for a control method and does not completely eliminate interference by mono- and trimethylamine. The authors have found that the interference by monomethylamine is due to the by-product formation of some nitromethane during the nitrous acid treatment. The procedure detailed herein eliminates the distillation as well as interference by the other amines in the concentrations normally encountered in crude reactor samples.

APPARATUS

A Model XX visible recording polarograph, manufactured by E. H. Sargent & Co., Chicago, Ill., was used. The polarographic cells were of the H-shaped type recommended by Lingane and Laitinen (*2*), one arm serving for the sample solution and the other for a saturated calomel cell anode. These cells were mounted in an air-agitated, vibration-insulated (*1*) water bath, thermostatically regulated to 25° ± 0.05° C. The capillary passed 1.366 mg. of mercury per second and the average drop time, over the −0.6- to −1.2-volt range and in the supporting electrolyte, of the analysis was 4.33 seconds, giving an $m^{2/3}t^{1/6}$ constant of 1.57 $mg.^{2/3}$ $second^{-1/2}$.

REAGENTS

Sulfamic Acid (NH_2HSO_3). Dissolve 15 grams in water to 100 ml.

Sodium Hydroxide. 30% by weight.

Sodium Hyposulfite. Commonly known as hydrosulfite, $Na_2S_2O_4$. High grade commercial material is satisfactory.

Iodine. Approximately 0.2 *N*, prepared by dissolving 50 grams of potassium iodide and 26 grams of iodine in 50 ml. of water and diluting to 1 liter. This solution need not be standardized.

Gelatin. 0.5 gram of Knox's No. 1 gelatin dissolved in 50 ml. of warm water. This should be freshly prepared daily.

PROCEDURE

The sample should be in aqueous solution containing 15 to 25% of total bases. Transfer an aliquot, not exceeding 5 ml. and containing 0.05 to 0.13 gram of dimethylamine, to an 8-inch test tube, dilute to 5 ml., and set in an ice bath. Add 3.0 ± 0.05 grams of sodium nitrite and 5 ml. of glacial acetic acid, and place in a water bath at 25° ± 2° C. for 10 minutes. Immerse the tube to within about 2 inches (5 cm.) of the top in an ice bath and, while swirling vigorously, add dropwise from a pipet sufficient sulfamic acid solution (about 17 ml.) to destroy the excess nitrous acid as indicated by test with starch–potassium iodide paper. An excess of sulfamic acid will do no harm. Add a drop of phenolphthalein indicator solution, titrate to neutrality with the sodium hydroxide solution, and add 0.1 ml. excess. Establishment of the correct alkalinity at this point is vital. Immerse the tube in a water bath at 60° C. and after it has come to temperature add 1.0 ± 0.05 gram of sodium hydrosulfite. Stopper the tube, invert it several times to dissolve the hydrosulfite completely, return it to the 60° C. bath for 5 minutes, cool to 25° C., and dilute to 100 ml. in a volumetric flask. Pipet 10 ml. into a beaker, add 50 ml. of water, 1 ml. of glacial acetic acid, and 1 ml. of starch indicator solution, and titrate to an exact end point with the iodine solution to destroy the excess hydrosulfite. Add 2.5 ml. of concentrated hydrochloric acid and 1 ml. of gelatin solution, and dilute to 100 ml. in a volumetric flask.

Electrolyze in the customary manner, using an initial potential of −0.3 volt and a sensitivity setting to give a step height of 100 to 200 mm. The curve is well defined, and, although the top plateau is inclined at a much greater angle to the horizontal than is the condenser current line, it is reproducible and accurately measurable. The final solution is stable and may be retained even overnight before polarographing.

EXPERIMENTAL

Preparation of Pure Materials. Anhydrous mono-, di-, and trimethylamines of 97 to 99% purity were dissolved in water, acidified with a slight excess of hydrochloric acid, evaporated until crystals began to form, cooled slowly to 5° C., filtered, washed with a mixture of 3 volumes of acetone to 1 of ethyl alcohol, and dried at 100° C. Further purification of each was then effected as follows:

Monomethylamine. Crystallized three times from denatured alcohol (2B formula, 95% ethyl alcohol plus 0.5% benzene).

Dimethylamine. Crystallized twice from a 1 to 1 by volume acetone–ethyl alcohol mixture.

Trimethylamine. Crystallized once from ethyl alcohol and twice from 1 to 1 acetone-alcohol.

All were washed after each filtration with a mixture of 5 volumes of acetone to 1 of alcohol. They were finally dried at 100° C. and stored in paraffin-sealed bottles; they are all somewhat hygroscopic. These products gave no test for ammonia with Francois reagent and purities ranging from 99.9 to 100.2% by chlorine and total base determination, and, in the case of monomethyl, by the Van Slyke (*4*) method. They were used in this form in the experimental work, not as the free bases.

Ammonia. Analytical reagent grade ammonium chloride was used without further purification.

Preliminary runs made in tetramethylammonium chloride showed that the reduction potentials of these amines are too close together for quantitative separation, which is in agreement with published data (*3*). Treatment of dimethylamine with carbon disulfide and sodium hydroxide to form dimethyldithiocarbamate produced a solution that yielded two polarographic waves, one at $E_{1/2}$ = −0.77 volt and the other at −1.35 volts. These were later shown to be due, respectively, to some reaction product of carbon disulfide, possibly trithiocarbonate, and to carbon disulfide itself. A usable wave from dithiocarbamate was not found, even in the absence of excess carbon disulfide.

Attention was then directed to the reaction with nitrous acid which destroys ammonia and monomethylamine, forms the nitroso derivative of dimethylamine, and has been stated not to react with trimethylamine. Introductory experiments on dimethylamine led to the following observations:

1. Nitrosation in mineral acid is prohibitively slow, but is rapid in acetic acid.
2. Dimethylnitrosamine is not reduced polarographically in neutral or alkaline solution but produces a definite wave in acid. The plateau is not parallel to the condenser current line but in-

Table I. Alkaline Reduction of Nitromethane

$Na_2S_2O_4$, Gram	5 *N* NaOH, Ml.	Temp., ° C.	Approx. pH	Step Height, Mm.
0.00	0.00	25	...	88
0.25	0.50	25	12	27
0.50	0.50	25	12	15
0.75	0.50	25	12	14
1.00	0.50	25	12	3
1.00	0.20	25	10	5
1.00	0.75	25	12+	32
1.00	1.00	25	13+	73
1.00	0.20	40	10	1
1.00	0.30	40	11	1
1.00	0.50	40	12	4
1.00	0.70	40	13	47

clined to it at angles of 30° to 40°, depending upon the height of the wave and other factors. Change in acid concentration or substitution of methanol or dimethylformamide for some of the water as solvent affect the plateau angle but little. The inclined plateau is, however, reproducible and accurately measurable.

3. Excess nitrous acid must be destroyed before electrolyzing, since it produces an interfering wave. Sulfamic acid is better for this purpose than urea and causes no interference.

Study of the nitrosation of dimethylamine revealed that for complete reaction at room temperature or below in a reasonable time, the concentration of acetic acid must be 50% by volume, or greater, and that at least 2 grams of sodium nitrite are required for 0.1 gram of base. When these conditions were applied to monomethylamine, a definite wave was unexpectedly obtained at $E_{1/2} = -0.78$ volt; the corresponding value for dimethylamine under the same conditions was -0.94 volt. The reaction of monomethylamine with nitrous acid is supposed to produce only methanol, water, and nitrogen. A relatively stable intermediate compound (nitroso, diazo, etc.) seemed highly improbable, but to confirm this point, a known weight of monomethylamine hydrochloride was analyzed by the Van Slyke method (*4*); a quantitative recovery of nitrogen was obtained. The solution remaining from this determination was treated with sulfamic acid and polarographed. Again the wave at $E_{1/2} = -0.78$ volt was obtained, proving the presence of some side reaction product and not a nitrogenous intermediate which would prevent quantitative recovery of elemental nitrogen. The most likely explanation appeared to be the presence of nitromethane, formed through a free radical mechanism. To test this hypothesis, a polarogram was made, under the same conditions, of a known sample of nitromethane. A sharply defined wave was produced at $E_{1/2} = -0.79$ volt, which was accepted as sufficient confirmation.

The nitromethane wave is close enough to that of dimethylnitrosamine to interfere. Numerous attempts were made to modify the conditions of nitrosation in such a way that the formation of this by-product would be prevented, but wide variations in acid type (hydrochloric, formic, propionic) and concentration, reaction time, and temperature had negligible effect. The addition of nonaqueous solvents, alcohols, dimethylformamide, and dioxane did not improve matters. The presence of free radical suppressors (diphenylamine, phenothiazine, *tert*-butyl catechol) had no effect, nor did traces of various metallic salts exert any negative catalytic effect. The respective boiling points of nitromethane and dimethylnitrosamine are about 101° and 150° C.; thus, elimination of the former by aeration or boiling without appreciable loss of the latter is impossible. Extraction also failed because no solvent was found that would dissolve one and not the other.

Differential reduction was next explored. In acid solution with titanous chloride, stannous chloride, or zinc dust, unsatisfactory results were obtained, but in an alkaline medium sodium hydrosulfite ($Na_2S_2O_4$) was found to reduce nitromethane without appreciable effect upon dimethylnitrosamine. To establish the optimum conditions of reduction, 1-ml. aliquots of 1 *M* aqueous nitromethane solution were diluted to 25 ml. in 8-inch test tubes, and various quantities of 5 *N* sodium hydroxide and solid hydrosulfite were added, allowed to react for 5 minutes, and diluted to 100 ml. Ten-milliliter aliquots were then diluted to 75 ml. and 1 ml. each of acetic acid and starch indicator were added; the solution was then titrated with iodine solution, acidified with 2 ml. of hydrochloric acid, diluted to 100 ml., and electrolyzed (Table I).

A blank on the reagents gave a step height of 1 mm.

These results show that the alkali concentration is critical and that a minimum of 1 gram of hydrosulfite is required. Later experiments showed that the temperature can be raised to 60° C. without reducing a detectable amount of dimethylnitrosamine. When ammonia was substituted for caustic soda, the results were less favorable.

The sodium hydrosulfite must be substantially free of thiosulfate, because the tetrathionate formed from this impurity upon titration with iodine produces an interfering polarographic wave. The quality of the hydrosulfite can be established by running a blank upon the reagents used in the analysis. Since thiosulfate is one of the atmospheric oxidation products of hydrosulfite, the solution after reduction should be diluted to volume, aliquoted, and titrated with iodine without delay. The final solution is stable for at least 24 hours. Iodine oxidizes hydrosulfite to sulfate, not thiosulfate.

Calibration. Calibration data were obtained from purified dimethylamine hydrochloride by putting it through the analytical procedure previously given in detail. The assays listed in Table II are the total weights of equivalent base used (not hydrochloride), only one tenth per 100 ml. of which appears in the solutions electrolyzed. The runs were made at a sensitivity setting of 2-0 (0.172 microampere per mm.) with initial potential of -0.3 volt and span potential of 3.0 volts.

These values plot to a straight line which does not pass through the origin because they are not corrected for condenser current rise, about 3 mm.

Ammonia and Trimethylamine. Various mixtures of the purified hydrochlorides were analyzed. The weights shown in Table III are again the equivalent weights of the bases. The polarographic conditions were the same as in the calibration.

The first five experiments in this group represent much more than the normal variation in the composition of crude reactor samples, and under these conditions the recovery of dimethylamine is satisfactory, particularly when a large enough sample is taken to produce a step height above 100 mm. The remaining

Table II. Calibration Data

$(CH_3)_2NH$ Base Total, Mg.	Step Height, Mm. A	B	C	Av.
10.5	21.0	21.0	20.5	20.8
20.9	39.0	39.0	38.0	38.7
41.8	75.5	75.0	75.5	75.3
62.7	109.5	109.0	109.5	109.3
104.5	183.5	183.5	184.5	183.8

Table III. Analysis of Known Amine Mixtures

Amine Base, Mg. $(CH_3)_2NH$	NH_3	CH_3NH_2	$(CH_3)_3N$	Step Height, Mm.	$(CH_3)_2NH$ Found Mg.	% recovery
20.9	10	10	30	38.5	21.2	101.5
41.8	60	40	120	74.5	42.3	101.2
62.7	10	30	120	109.5	62.9	100.3
83.6	80	80	80	146.0	84.3	100.7
104.5	100	100	100	179.0	103.6	99.3
20.9	500	...	...	36.5	20.0	95.8
20.9	1000	...	...	32.5	17.5	83.8
20.9	...	500	...	38.0	20.8	99.6
20.9	...	1000	...	27.0	14.4	69.4
20.9	...	...	500	40.0	22.0	105.3
20.9	...	...	1000	40.5	22.3	106.8

Table IV. Analysis of Commercial Samples

Sample	Assay, Gram	Step Height, Mm.	$(CH_3)_2NH$ Found Gram	%
H	0.955	161.0	0.0931	9.8
H	0.955	163.0	0.0943	9.9
H	0.955	159.0	0.0920	9.6
J	0.470	169.5	0.0981	20.9
J	0.470	170.5	0.0987	21.0
J	0.470	167.5	0.0969	20.6
3	0.146	155.5	0.0900	61.6
3	0.146	156.5	0.0905	62.0
3	0.146	155.5	0.0900	61.6
D	0.0660	99.0	0.0567	85.9
D	0.0660	101.5	0.0572	86.7
D	0.0660	99.0	0.0567	85.9

six experiments are included to indicate the limitation of the method. In the presence of very large amounts of ammonia and monomethylamine, the recovery is low, probably because too much of the nitrous acid is consumed in their decomposition and not enough remains for complete nitrosation of the dimethylamine. Doubtless, an increase in the amount of nitrite would improve the recovery, but this point was not studied because it was beyond the range of the present investigation. High concentrations of trimethylamine, on the other hand, produce high results owing to nitrosation of some trimethylamine. No conditions for the nitrite reaction were found that would prevent this and still give quantitative reaction with the dimethylamine.

As a final test of the method, numerous samples of commercial material of widely varying composition were analyzed; typical results are set forth in Table IV.

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Interferometry in Electrophoresis

L. G. LONGSWORTH
The Rockefeller Institute for Medical Research, New York, N. Y.

The precision of the schlieren methods for the measurement of the refractive index changes that occur in the analysis of protein mixtures by the Tiselius method is limited by diffraction effects arising at the schlieren diaphragm. In order to increase the precision with which the analyses can be made, a modification of the Rayleigh interferometer, suggested by Philpot and Cook and by Svensson, has been adapted, with only minor changes in existing equipment, for use in electrophoresis. In routine work a precision of 2×10^{-6} in the relative refractive index is obtained and, under favorable conditions, 2×10^{-7}. This may be compared with the value of about 1×10^{-5} that characterizes the schlieren procedures. With the interference method it is possible to work with smaller quantities of material, an important consideration in the study of many biological substances, and at dilutions at which the disturbing effects of superimposed gradients at the boundaries are reduced.

PHILPOT and Cook (*9*) and, independently, Svensson (*11, 12*) have suggested a modification of the Rayleigh interferometer for the measurement of the refractive index changes at the boundaries that occur in diffusion, electrophoresis, and sedimentation studies. As described below, the modification requires a cylinder lens, and in testing the method for diffusion measurements (*8*) it was found that precise results will not be practicable until an adequately corrected lens of this type is available. In the case of an electrophoretic analysis, however, the aberrations of this lens, although distorting somewhat the boundary "shape," are not a source of error in the measurement of the difference of refractive index across a boundary. Even with the uncorrected lenses now available, a more precise analysis is possible with this interference method than with the schlieren procedures in current use.

PROCEDURE

The principle of the method is shown in Figure 1, in which *A* is a point source of monochromatic light, *L* is a lens that forms an image of this source in the plane at *P*, *M* is a plate with vertical slits that mask the boundary channel and a neighboring reference channel, respectively, and *C* is the cylinder lens, with its axis horizontal, that focuses the cell (not shown) vertically but does not affect the horizontal spacing of the Rayleigh fringes, *F*, that are formed at *P* by the vertical slits. If the fluid in the boundary channel is homogeneous, these fringes are vertical, as shown in Figure 1. With one or more boundaries in this channel, however, they are warped, as shown in the photomicrograph of Figure 2, *a*, into overlapping segments of the complete refractive index-height curve that intersect diagonally the central diffraction envelope. Owing to the relatively great length (47 mm.) of the fringe negative, it was necessary to enlarge it in the four sections shown here.

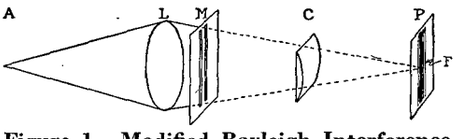

Figure 1. Modified Rayleigh Interference Method

In order to use the new procedure only minor changes in the electrophoresis equipment of this laboratory (*5*) are required. The lens, *L*, of Figure 1 will be recognized as the conventional schlieren lens and the plane, *P*, as that of the schlieren diaphragm which is replaced by a plate holder (*6*). Moreover, the cylinder lens that is used in the inclined slit method of schlieren observation may also be used at *C*. Superposition of a vertical slit upon the illuminated horizontal one that serves as a light source in the schlieren method reduces this to the "point" source, *A*. The conventional mask for the Tiselius cell, which contains a slit in front of each side of the channel and a flap exposing one side at a time, is replaced by one with a double slit for each side. One of these exposes the channel and the other the reference path through the adjacent thermostat fluid. Apparently, because of the inhomogeneity of the green line in the spectrum of the H4 lamp,

fringes are not obtained if the path difference is excessive. Thus it was necessary to modify the Tiselius cell by including in the reference path a thickness of glass equal to that of the channel windows. This was done by fusing glass strips, duplicates of the channel windows, to the outside wall of each channel. With water in the newest cell, of fused quartz, the windows of one channel are such that the fringes are straight to within 0.1 of their horizontal separation, d, of Figure 2, whereas in the case of the other channel the deviation is 0.3 d. Although this cell was used in the analyses reported in Table I, the patterns of Figure 2 were obtained in a borosilicate glass cell in which the deviations from linearity were somewhat greater. Each slit in the mask, M of Figure 1, is 1.5 mm. wide and the distance, δ, between their centers is 6 mm. With an optical distance, b', of 186 cm. from the mask to the photographic plate the horizontal separation, d of Figure 2, a, of the Rayleigh fringes is $b'\lambda/\delta$ or 169 microns.

In a semimicro electrophoresis equipment now under construction in this laboratory the silvered window of a 2 × 10 × 75 mm. channel reflects the light back through the cell and through an autocollimating schlieren lens placed between the source and the cell. Although the juxtaposition of the incident and reflected light makes it impracticable (*9*) to use the cylinder lens as shown in Figure 1, the Philpot-Svensson camera may be adapted (*12*) to form the fringes in its focal plane.

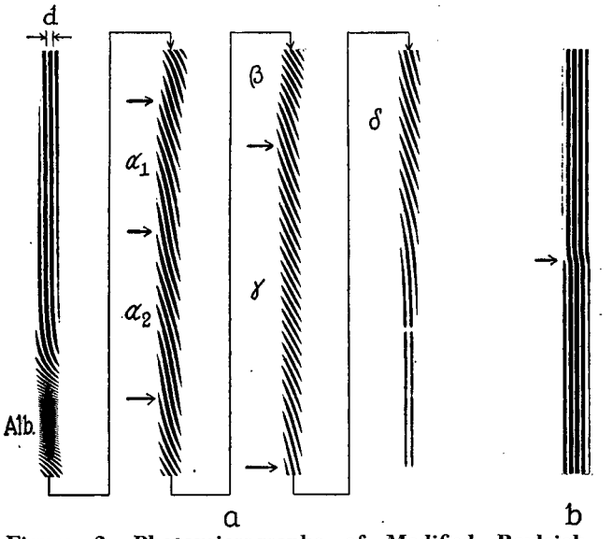

Figure 2. Photomicrographs of Modified Rayleigh Fringes Obtained on Electrophoresis

a. **Human serum (1 vol. serum + 5 vol. buffer)**
b. **0.001 *N* KCl–0.00064 *N* KIO_3 boundary

The fringe pattern of Figure 2, a, was obtained after electrophoresis of a human serum, at a total protein concentration of 1.18%, in a 0.1 N sodium diethylbarbiturate buffer of pH 8.6 for 14,500 seconds at 4.77 volts per cm. With the vertical fringes conjugate to the homogeneous column of buffer at the top of the channel aligned parallel to the comparator axis, and with the microscope cross hairs focused on a minimum, the reading, H, at each minimum, j, intersecting the envelope was tabulated. Although not used in the present investigation, in future work the alignment will be facilitated if the cell mask includes another pair of vertical slits, in the path of which a properly oriented glass plate is placed. A set of vertical fringes is then formed at P, Figure 1, that is parallel to, but displaced from, the envelope of those from the boundary channel. This use of a displacing plate to give an invariant frame of reference on the photographic plate is analogous to the procedure employed in work with Gouy fringes (*1*).

In Figure 3 the reciprocals, $1/\Delta H$, of the vertical separations between the minima of Figure 2, a, are plotted as ordinates against the mean fringe position, $\bar{H}$, as abscissas. Owing to the large number of fringes and the precision with which minima can be located, such a tabular differentiation reproduces closely the variation of the gradient of refractive index. In fact, the curve of Figure 3 is a tracing of the schlieren scanning photograph that was recorded in the same experiment, the coordinate scales being adjusted with the aid of the constants characteristic of the two optical systems. Because the area under each peak of the traced pattern corresponds closely to the refractive index difference determined interferometrically as described below, confirmation is thereby afforded of the correctness of the exposure and tracing procedures used in this laboratory in the interpretation of the schlieren scanning patterns. However, the slight distortion of boundary "shape" by the cylinder lens, although scarcely apparent on the scale of Figure 3, renders the interference method unsuitable for mobility measurements until corrected lenses are available.

For an analysis it is unnecessary, however, to prepare the plot of Figure 3. The position of a minimum in the gradient curve corresponds to a maximum in the fringe separation and the number, j, of the fringe at which it occurs can be determined by inspection of the differences, ΔH, or to a fractional part of a fringe by numerical interpolation. For example, the separations, ΔH, of the 43rd, 44th, 45th, and 46th fringe are 466, 532, and 493 microns, respectively, and ΔH is thus a maximum in the neighborhood of the 45th fringe. If these three values of ΔH are used to determine the constants of a parabola (*10*), the value of j at its apex is 44.6. In Figure 2, a, ΔH, is a maximum at j = 0, 44.6, 50.4, 56.7, 69.0, 92.0, and 102.0, this last interval representing the δ—i.e., protein concentration boundary. The positions of these maxima are indicated by the arrows in Figure 2, a. If all the serum proteins have essentially the same specific refraction, the apparent composition of this serum is 44.6/92.0 or 48.5% albumin, 6.3% α_1-, 6.8% α_2-, 13.4% β-,

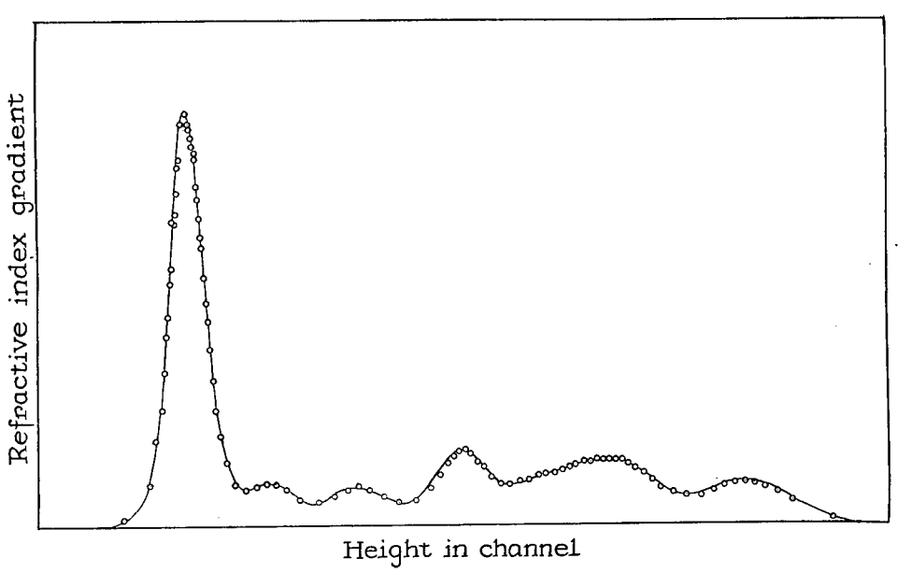

Figure 3. Electrophoretic Pattern of Human Serum at Total Protein Concentration of 1.2%

O Reciprocal of fringe separation
— Tracing of schlieren scanning pattern

Table I. Apparent Composition of Equimolar Mixtures of Aspartic and Glutamic Acids

Taken		Apparent Mole % of Aspartic Acid Found	
Acid concn., C_A, mole/1000 g. solution	Buffer salt concn., C_B, equivalent/liter	Descending	Rising
0.04	0.2	51.1	57.8
0.08	0.2	50.7	62.1
0.04	0.1	49.9	62.3
0.08	0.1	45.8	68.3

and 25.0% γ-globulin. Numerical interpolation for the value of j at a maximum in the fringe separation is a more objective procedure than the conventional location of a minimum in the schlieren scanning pattern (*4*).

The protein concentration of 1.18% represented by Figure 2, *a*, is about half of that ordinarily used with the schlieren method. With the present equipment, in which the cylinder lens of 412-mm. focal length reduces the channel height of 86 to 47 mm. at the plate, the usual protein concentration of 2% leads to about 40 fringes per mm. conjugate to the sharp albumin boundary. Although these fringes are clearly defined, the limit of the resolving power of most photographic emulsions is being approached. Below 1% protein, on the other hand, the density increments at the globulin boundaries may not stabilize them adequately against the disturbing effects of convection. For routine work with human serum and plasma a protein concentration of 1 to 2% is, therefore, suggested.

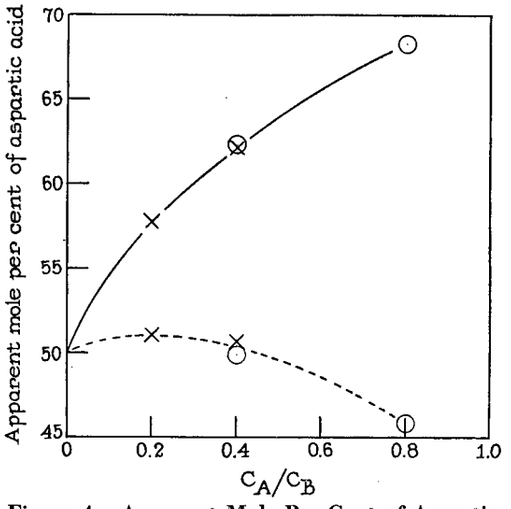

Figure 4. Apparent Mole Per Cent of Aspartic Acid in Equimolal Mixtures of Aspartic and Glutamic Acids

- - - - From pattern of descending boundaries
——— From pattern of rising boundaries
× 0.2 *N* sodium acetate buffer
○ 0.1 *N* sodium acetate buffer

The fringes intersecting the envelope in Figure 2, *a*, for example, are segments of the complete refractive index–height curve. Consequently, if the plate is shifted along the cross axis of the comparator by a known fraction, say $^1/_4$, of the spacing, d, between the vertical fringes the readings, H', for each minimum are then the values of H for $j + {}^1/_4$. In this way the data available for numerical treatment can be increased almost indefinitely without recourse to photometry or other estimates of optical density. The fringes afforded by this method thus appear to have an advantage over the horizontal ones that are obtained with a Jamin type of interferometer (*3*).

The precision inherent in the interference method doubtless arises from the greater accuracy with which the minima can be located as compared with the edges of the relatively coarse diffraction patterns that are encountered in using the schlieren method. The fringe displacement at a boundary such as that of Figure 2, *b*, can be measured to within 1 or 2 microns. With d = 169 microns this corresponds to a path difference of 0.01 wave. With λ = 5461, and in a 2.5-cm. channel, 0.01 wave is equal to 2 $\times 10^{-7}$ in the refractive index, n. In Figure 2, *b*, the arrow indicates the position of a boundary across which $\Delta n = 77 \times 10^{-7}$, the fringe displacement here being 60 microns.

APPLICATION

A possible application of the interference method that takes advantage of its precision is the analysis of mixtures of amino acids and peptides. Owing to the relatively low equivalent weight of these solutes, the available theory (*7*, *13*) indicates that the apparent composition of such a mixture would be expected to deviate significantly from the true value. The interference method may, however, permit analyses at such dilutions as to justify extrapolation of the apparent composition to zero concentration of the solute. As an example, an equimolar mixture of aspartic and glutamic acids has been analyzed at total acid concentrations of 0.08 and 0.04 moles per 1000 grams of solution in a 0.2 and a 0.1 *N* sodium acetate buffer solvent of pH 4.6. The results are given in Table I and Figure 4, where account has been taken of the unequal refractive increment, per mole, of these two acids. In the 0.1 *N* buffer at 1°, with a 2.50-cm. path and mercury green light, the increment for 0.04 molal glutamic acid is 46.2 fringes, whereas for aspartic acid at the same concentration it is 40.8 fringes.

As is shown in Figure 4, the results at the two buffer salt concentrations fall approximately on the same curve if the ratio, C_A/C_B, of the acid concentration to that of the buffer salt is taken as abscissa. At C_A = 0.04 an error of 0.1 fringe corresponds to one of 0.5% in the apparent composition. Although the apparent values from the patterns of the descending boundaries are much nearer the true composition than from the rising boundaries, it is disturbing that neither pattern permits a linear extrapolation to the true value of 50%. This indicates the need for a further development of the Dole theory (*2*) to provide adequate extrapolation formulas. In view of the relatively large equivalent weights of most proteins, it is clear from Figure 4 that linear extrapolation of the apparent composition of their mixtures is probably justified.

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Colorimetric Determination of 2-Nitro-1,1-bis (*p*-Chlorophenyl)alkanes

LAWRENCE R. JONES AND JOHN A. RIDDICK
Commercial Solvents Corp., Terre Haute, Ind.

2-Nitro-1,1-bis(*p*-chlorophenyl)alkanes are the active constituents of a new insecticide. This study was undertaken to develop a method of analysis for the amount of insecticide residues in fruits, vegetables, and plant foliage, and in animal tissues. The colorimetric method described will quantitatively determine the insecticide in amounts as low as 50 micrograms per sample with an error of not more than 10 micrograms. The error decreases to 5 micrograms as the amount of insecticide increases to about 100 micrograms. Amounts as low as 10 micrograms per sample may be detected. This method is specific for nitroalkanes. It may be used for the determination of insecticide residues and for the study of penetration, translocation in vegetable tissues, distribution in animal tissues, and excrets in toxicity studies.

DILAN is the trade-mark used for the insecticide containing 2 parts of technical 2-nitro-1,1-bis(*p*-chlorophenyl)butane and 1 part of technical 2-nitro-1,1-bis(*p*-chlorophenyl)propane. Its technical components have been tested for their insecticidal properties under the following code designations: 2-nitro-1,1-bis(*p*-chlorophenyl)butane as CS-674A, and 2-nitro-1,1-bis(*p*-chlorophenyl)propane as CS-645A. Dilan and each of its components have been shown to be particularly effective for the control of the Mexican bean beetle.

Infrared and polarographic methods have been developed for the analysis of Dilan and its components. The infrared method is not sufficiently sensitive for the determination of Dilan in spray residues or in biological tissues. The polarographic method has the required sensitivity, but extracted materials from both plant and animal tissues interfere with the method.

The two principal components of Dilan contain an aliphatic nitro group which offers a means of specific characterization from other commonly used insecticides. Several colorimetric methods have been proposed for the analysis of nitroparaffins (*1, 2, 4, 5, 7*). The method developed by Scott and Treon (*7*) appeared to offer the best possibility for the precise determination of small amounts of an aliphatic nitro compound. A preliminary study indicated that this method probably could be used as a basis for an accurate method for Dilan in micro amounts in vegetable and animal tissues.

The method is based on the fact that a nitro group attached to a primary or secondary alkane carbon exists in equilibrium with its tautomeric aci- form: $R_2CHNO_2 \leftrightarrows R_2C{=}NO_2H$. The equilibrium is shifted to the right in the presence of an alkali (*3*). The aci-nitroalkane forms a colored complex with ferric chloride. Lowry and Magson (*6*) found that the isomerization of nitrocamphor took place in nonaqueous media and that 1% of water in ethyl alcohol solutions would roughly double the speed of reaction. The work of Scott and Treon (*7*) indicates that nitroparaffins are quantitatively changed to the aci- form in aqueous solution.

2-Nitro-1,1-bis(*p*-chlorophenyl)butane and 2-nitro-1,1-bis(*p*-chlorophenyl)propane are insoluble in water, but may be handled conveniently in organic solvents. This method is based on the formation of the aci-nitroparaffin in organic solvents instead of water.

EXPERIMENTAL

The factors influencing the formation of the aci- form and the ferric complex, the intensity of color of the ferric complex, and stability of the color were studied. It was found that methyl or ethyl alcohols were satisfactory solvents, provided some water was present. Briefly, the procedure used for examining the influence of the several factors was as follows:

An ethyl alcohol (S.D. Formula 3A, anhydrous) solution containing 1 mg. of the nitro compound per milliliter was transferred to a blood sugar tube, and the nitro group was converted to the aci- form with methanolic sodium hydroxide. The colored complex was developed by the addition of hydrochloric acid followed by ferric chloride solution. The color intensity was measured on a Beckman Model DU spectrophotometer.

Absorption Spectrum. The determination of spectral-transmittance curves showed that the red iron complex resulting from the reaction of purified and technical 2-nitro-1,1-bis(*p*-chlorophenyl)butane and 2-nitro-1,1-bis(*p*-chlorophenyl)propane, and Dilan has a maximum absorption at 490 mμ. Scott and Treon (*7*) found the maximum absorption for the simple nitroparaffins in aqueous solution under otherwise similar experimental conditions to be 500 mμ.

Stability of Colored Complex. Pure and technical Dilan were treated to develop the colored complex. The transmittancy of each sample was determined at time intervals. The color was found to be stable for at least one hour.

Scott and Treon (*7*) found that the simple secondary nitroparaffins reacted with the reagents to produce the colored complex, which faded rapidly. A sample of 2-nitropropane was tested by the basic procedure hereinafter described. The color developed and slowly faded from 4 to 10% transmittancy within an hour. It is believed that simple secondary nitroparaffins may be determined in an essentially alcoholic system by reading the transmittancy at a fixed time after color development. Apparently, the simple secondary nitroparaffin colored complex is more stable in alcoholic solvents than in water. The increased stability of the secondary 2-nitro-1,1-bis(*p*-chlorophenyl)alkanes may be due to the presence of the phenyl groups.

Effect of Water on Colored Complex. When anhydrous reagents were used, very little of the colored iron complex was formed. The intensity of the color increased to a maximum as the amount of water was increased and then decreased. The optimum amount of water was found to be 2.0 ml. in 12.5-ml. total volume. The exact volume of water may be varied from 1.5 to 2.5 ml. without an appreciable change in the color intensity; however, the amount of water must be kept constant to obtain the best accuracy. The effect of water on the color intensity is shown by the data in Table I. The optimum amount of water has been incorporated in the ferric chloride reagent.

Effect of Acidity on Intensity of Color. One milliliter of 0.500 *N* methanolic sodium hydroxide solution was added to tubes containing 2 mg. of Dilan in 5 ml. of methanol. After 10 minutes varying amounts of 0.500 *N* methanolic hydrochloric acid were added. Two milliliters of aqueous ferric chloride solution were added to each tube, and the transmittancy was determined using a blank prepared with the same amounts of reagents. The results are shown in Table II.

The maximum color intensity was obtained in an alcoholic solution which was 0.004 *N* with respect to hydrochloric acid. Scott and Treon (*7*) found the greatest color intensity when the concentration of hydrochloric acid was about 0.04 *N*. An excess of acid corresponding to 0.008 *N* in the final solution was chosen to compensate for small errors that may be made when preparing or adding the reagents. This small increase in acidity will not change materially the sensitivity of the method.

Sodium hydroxide and ferric chloride reagents must be made fresh whenever one or the other of the reagents gives out and a calibration curve must be run for the new reagents. This procedure assures the maximum accuracy.

Reaction Time and Temperature. It was found that these factors were not critical. The reaction proceeds rapidly at room temperature. A reaction time of 10 minutes was chosen for convenience.

Transmittancy and Concentration. Calibration curves were determined for both purified and technical 2-nitro-1,1-bis-(*p*-chlorophenyl)butane, 2-nitro-1,1-bis(*p*-chlorophenyl)propane, and Dilan by plotting concentration against optical density. The six materials gave a straight-line calibration originating at zero concentration and optical density. The color obeys Beer's law for amounts to 3.5 mg. of Dilan.

Table I. Effect of Water on Color Intensity

Water in 12.5 Ml. of Reaction Mixture *Ml.*	Transmittancy %
0.0	82.0
0.4	30.6
0.6	24.1
0.8	18.8
1.0	17.2
1.6	14.2
2.0	13.3
2.4	14.0
3.0	15.8
4.0	16.8

Table II. Acid *vs.* Color Transmittancy

0.500 *N* Acid Added *Ml.*	Concn. of HCl in Final Solution *N*	Transmittancy %
1.00	0.00	16.0
1.10	0.004	15.2
1.20	0.008	16.2
1.30	0.012	17.0
1.40	0.016	18.5
1.50	0.020	19.6
1.60	0.024	20.5
1.70	0.028	22.2
1.80	0.032	24.0
1.90	0.036	25.1
2.00	0.040	27.1
2.10	0.044	28.6
2.20	0.048	30.5
2.30	0.052	31.8
2.40	0.056	32.6
2.50	0.060	35.8

Table III. Recovery of Dilan Added to Green Beans

Sample *G.*	Dilan Added *Mg.*	Dilan Recovered *Mg.*	Dilan Recovered %
100	0.00	0.00	...
500	0.00	0.00	...
500	0.50	0.48	96
500	0.50	0.49	98
500	0.050	0.050	100
500	1.00	0.97	97
100	1.00	1.00	100
100	1.00	0.98	98
100	0.50	0.51	102
100	0.50	0.49	98
100	0.050	0.050	100
100	0.10	0.09	90
100	0.050	0.042	84
100	0.050	0.050	100
100	0.050	0.050	100
100	0.050	0.052	104

Table IV. Recovery of Dilan Added to Rabbit Tissue

Type of Tissues	Weight of Tissue *G.*	Dilan Added *Mg.*	Dilan Recovered *Mg.*	Dilan Recovered %
Liver	5.0	1.00	0.95	95
Liver	5.0	1.00	0.96	96
Liver	5.0	0.50	0.50	100
Liver	5.0	0.50	0.48	96
Muscle	5.0	1.00	0.97	97
Muscle	5.0	2.00	1.95	97
Fat	5.0	10.00	9.75	98
Fat	1.0	3.00	3.00	100
Fat	0.5	2.00	1.90	95

APPARATUS

Soxhlet extractor.
Spectrophotometer, Beckman, Model DU.
Steam bath.
Blood sugar tubes, Lewis-Benedict, graduated at 12.5 and 25 ml.
Tissue grinder, borosilicate glass, Scientific Glass Apparatus Co., No. J-3610, or equivalent.
Waring Blendor.

REAGENTS

Ethyl alcohol, anhydrous, S.D. Formula 3A, or anhydrous methanol.
Ethyl ether, reagent grade.
n-Hexane, Phillips Petroleum Co., commercial grade, redistilled.
CS-645A, and the purified compound, CS-645.
CS-674A, and the purified compound, CS-674.
Dilan standard, prepared from 2.000 parts of purified CS-674 and 1.000 part of purified CS-645. The CS-645 and CS-674 were estimated to be at least 99.0% pure on the basis of partition chromatographic, infrared, and polarographic analyses and solubility data.
Methanolic sodium hydroxide, 0.500 *N*.
Ferric chloride reagent, 10 grams of ferric chloride, $FeCl_3 \cdot 6H_2O$, dissolved in sufficient water to make 2000.0 ml. of solution and mixed with 1000.0 ml. of 0.600 *N* methanolic hydrochloric acid.

BASIC PROCEDURE

Preparation of Calibration Curves. Prepare a solution of Dilan standard in ethyl alcohol to contain 1 mg. per milliliter. Use the solution of Dilan in ethyl alcohol immediately, because the Dilan apparently is not stable in alcoholic solution for an extended period of time. To five Lewis-Benedict tubes add 0.0, 0.5, 1.0, 2.0, and 3.0 ml., respectively, of the standard Dilan solution.
Dilute each to 7.0 ml. with ethyl alcohol.
Add 1.0 ml. of the methanolic sodium hydroxide solution, mix thoroughly, and allow to stand at room temperature for 10 minutes.
Add 3.0 ml. of the ferric chloride reagent.
Dilute to 12.5 ml. with ethyl alcohol and mix.
Transfer the clear, colored solution to a 1-cm. Corex cell and read the transmittancy at 490 mμ with the spectrophotometer, using the 0.0 prepared standard as a blank for the 100% transmittancy setting of the instrument.
Plot concentration *vs.* transmittancy on semilog graph paper.
Analysis. Make the analysis in the same manner as described above, but prepare the sample so that an alcoholic aliquot of 7 ml. or less does not contain more than 3.5 mg. Prepare a blank for each series of determinations and use it to set the spectrophotometer at 100% transmittancy.

APPLICATION

Determination of Dilan in Plant and Animal Tissues Containing No Fat. The plant and animal tissue containing approximately 0.1 to 2 mg. of Dilan were prepared for analysis by pulverizing the tissue. The plant tissue was pulped in a Waring

Blendor with or without the aid of a small portion of *n*-hexane. The animal tissue was ground in a mortar, or tissue grinder. Each sample was then extracted for 1 hour with 250 ml. of *n*-hexane in a Soxhlet extractor. The extract was transferred to a beaker, covered with a ribbed watch glass, and evaporated just to dryness. While still warm, the residue was dissolved and quantitatively transferred to a Lewis-Benedict tube with a total volume of alcohol not exceeding 7.0 ml. This solution was used for analysis by the proposed method.

If the colored solutions were turbid, they were centrifuged or filtered prior to the color estimation.

It was found that *n*-hexane residue contained a small amount of color that interfered with the determination. Blanks were prepared for the 100% transmittancy setting by evaporating 250 ml. of *n*-hexane in a beaker, dissolving the residue in not more than 7 ml. of alcohol, and proceeding as directed for preparation of the calibration curve. This procedure eliminated the error caused by the solvent residue.

Determination of Dilan in Animal Tissues Containing Fat. Animal tissue containing fat yielded a turbid solution that could not be clarified either by filtering or centrifuging. It was found that the solution could be clarified by adding 5 ml. of ether after the addition of the ferric chloride reagent. The solution was diluted to 25 ml. instead of 12.5 ml. with alcohol. A calibration curve was prepared for the determination of Dilan in animal tissues containing fat using the same modification, and blanks for the 100% transmittancy setting were likewise modified.

RESULTS

An alcoholic solution of Dilan of known concentration was evenly spaced by dropping on green beans and rabbit tissue. The beans and rabbit tissue were allowed to stand varying lengths of time from one hour to one day, and were then analyzed for Dilan by the method given above. Typical data are presented in Tables III and IV.

DISCUSSION

An alternative procedure for the recovery of Dilan from green beans consisted of successively extracting (also called stripping) a weighed sample of the treated beans with three 100-ml. portions of *n*-hexane in a separatory funnel. By this extraction procedure, recoveries of Dilan were obtained which were equal to those obtained by the longer described procedure.

Dilan added to animal tissue which was ground with anhydrous sodium sulfate to ensure dehydration of the tissue could not be recovered even on prolonged extraction with *n*-hexane.

The following conclusions were drawn from a study of the results of analyses of several hundred samples of animal tissue:

There is an average error of +8 micrograms for tissues from animals used as controls. This error probably is due to a combination of three factors: error in setting the instrument at 100% transmittancy from day to day, error in reading the transmittancy from day to day, error in reading the transmittancy and calibration curve approaching 100% transmittancy, and difference in extracted color from different tissues and different amounts of the same type of tissue.

The precision and accuracy of the method decrease rapidly as the Dilan content of the sample decreases below 50 micrograms.

The authors consider a result less than 50 ± 10 micrograms as qualitative, and a result of 10 micrograms or less as zero. If 10 to 50 micrograms are found, a larger sample should be used or the result should be reported as less than 50 micrograms.

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Analytical Use of the Formation of the Beryllium-Fluoride Complex

FRITZ FEIGL AND A. SCHAEFFER

Laboratorio da Produção Mineral, Ministerio da Agricultura, and

Laboratorio Quimico da Escola Tecnica do Exercito, Ministerio da Guerra, Rio de Janeiro, Brazil

Beryllium ions have such a great tendency to form stable complex BeF_4^{--} ions that beryllium nitrate solutions are able to dissolve insoluble fluorides and to demask reaction systems which are masked by fluoride. Beryllium silicates and metallic beryllium are transformed into potassium fluoberyllate by fusing or sintering with potassium hydrogen fluoride. These effects permit essential simplifications in gravimetric analysis of fluorspar and cryolite, and detection of phosphate, molybdate, tungstate, iron, and titanium in presence of an excess of fluoride and of beryllium in minerals, ores, and alloys.

IT HAS been reported (*7*) that fluorspar suspended in dilute acids is completely dissolved upon addition of complex formers of fluorine: aluminum, iron, zirconium, and beryllium ions, as well as boric acid. When beryllium nitrate or chloride is used, the calcium can be precipitated as oxalate, after solution in acid and buffering. New experiments have shown that calcium fluoride, produced by precipitation, is completely dissolved, and finely powdered fluorspar is almost completely solubilized even in the absence of acids, merely by heating with concentrated solutions of beryllium nitrate. Obviously this is due to the elimination of fluorine ions from the solution equilibrium of calcium fluoride (Equation 1) through formation of complex fluoberyllate (BeF_4^{--}) ions according to Equation 2.

$$CaF_{2(solid)} \longrightarrow Ca^{++} + 2F^- \qquad (1)$$

$$4F^- + Be^{++} \longrightarrow BeF_4^{--} \qquad (2)$$

$$2CaF_2 + Be^{++} \longrightarrow BeF_4^{--} + 2Ca^{++} \qquad (1) + (2)$$

Similar displacement of equilibria is produced by aluminum, iron, and zirconium ions, due to the formation of complex AlF_6^{---}, FeF_6^{---}, and ZrF_6^{--} ions. Boric acid eliminates fluorine ions by formation of BF_4^- ions (*2*, *11*). In addition to calcium fluoride,

the insoluble fluorides of thorium, cerium, lanthanum, lead, and magnesium are dissolved by beryllium nitrate in excess. Therefore these fluorides cannot be precipitated from beryllium-containing alkali fluoride solutions.

Because the small concentration of fluorine ions in water suspensions of acid-resistant fluorides is sufficient to produce fluoberyllate ions, the same is to be expected from the small fluorine ion concentration in water solutions of compounds in which fluorine, owing to complex or principal valence binding, is part of a stable anion. This really is true, and means that the following gross reactions are valid:

$$2AlF_6^{---} + 3Be^{++} \longrightarrow 3BeF_4^{--} + 2Al^{+++} \quad (3)$$

$$MoO_2F_4^{--} + Be^{++} + 2H_2O \longrightarrow MoO_4^{--} + BeF_4^{--} + 4H^+ \quad (4)$$

According to Equations 3 and 4, reactions of aluminum and molybdate ions, which are masked in the presence of fluoride ions, occur immediately after addition of a sufficient amount of beryllium ions (*5*). The same demasking takes place in the solution of other compounds of the above type.

The solubilization of insoluble fluorides and the demasking in solutions of fluorine-containing principal and complex compounds by beryllium salts are due to the great stability of fluoberyllate ions. It is therefore remarkable that the color lake reaction of beryllium with quinalizarin described by Fischer (*9*) occurs also in solution of potassium fluoberyllate. All other metals, however, which form color lakes with quinalizarin, are masked when present in the form of normal or complex fluorides. Fischer has used the sintering of beryllium-containing silicates with sodium silicofluoride, thus transforming metals into fluorides and complex fluorides, in order to determine beryllium colorimetrically through the quinalizarin reaction. The formation of potassium fluoberyllate can also be obtained by sintering with potassium hydrofluoride, permitting thus, in combination with the quinalizarin reaction, a sensitive and specific test for beryllium in minerals, ores, and alloys. In the following, the analytical use of the formation of the beryllium-fluoride complex is described.

GRAVIMETRIC DETERMINATION OF CALCIUM IN FLUORSPAR

The analytical literature does not show whether the above-mentioned solubilization of calcium fluoride by beryllium nitrate has been considered in the analysis of fluorspar. Since 1941, in the Laboratories of Mineral Production of the Ministry of Agriculture, Rio de Janeiro, all fluorspar analyses have been executed in the following manner:

Procedure. From 0.1 to 0.2 gram of the finely powdered material is treated with 0.1 *N* acetic acid for 30 minutes upon a water bath, and then filtered. The amount of calcium in the filtrate, determined by precipitation as calcium oxalate, indicates the "acid-soluble" calcium. The residue together with the filter paper is dried and calcined and then quantitatively transferred to a beaker where it is treated with 5 to 10 ml. of 2 *N* hydrochloric acid, 50 ml. of water, and 1 to 2 grams of beryllium nitrate. After heating for about 10 minutes any residue is filtered, dried, and calcined (insoluble residue). The filtrate from this residue contains, besides beryllium fluoride and an excess of beryllium nitrate, the whole calcium formally bound with fluoride. This solution is treated with ammonia until a small amount of precipitate remains; then the precipitate is dissolved by dropwise addition of hot dilute acetic acid and calcium oxalate is precipitated and determined in the usual way. The amount of fluoride corresponding to this calcium value indicates the fluorine content of the material under examination.

Instead of determining calcium by weighing the oxalate or by titrating the calcium-bound oxalic acid with permanganate, the following can be recommended:

The precipitated calcium oxalate is filtered after standing overnight and washed with dilute ammonium oxalate solution. After drying and calcination to calcium oxide, the latter—which always contains calcium carbonate—is dissolved in a given amount of 0.1 *N* hydrochloric acid, and the excess acid is back-titrated with 0.1 *N* sodium hydroxide, using methyl orange as indicator. This procedure has the advantage that the precipitated calcium oxalate does not require washing with water, with its attendant small but inevitable losses. Any ammonium oxalate retained by calcium oxalate is completely eliminated by calcination. Adsorbed beryllium salt is harmless, because beryllium oxide formed by glowing is not attacked by diluted acids.

In order to illustrate this procedure, calcium determinations were carried out with pure natural fluorspar crystals; in amounts of 0.1 to 0.2 gram the product was dissolved in acid beryllium nitrate solution without leaving a visible residue.

Fluorspar, G.	Ca, G.	Ca, %	F Calcd., %
0.1068	0.0547	51.23	48.57
0.0941	0.0481	51.11	48.45
0.1048	0.0535	51.06	48.41
0.1007	0.0519	51.54	48.87
0.0994	0.0511	51.41	48.74
		Av. 51.27	48.61
		Theoretical 51.33	48.67

GRAVIMETRIC DETERMINATION OF ALUMINA IN CRYOLITE

The hitherto used procedure for gravimetric determination of alumina in cryolite (Na_3AlF_6) consists in fuming the material with concentrated sulfuric acid, dissolving the residue in water, and precipitating alumina by a known procedure. The following method of destroying the complex alumina fluoride and precipitating aluminum oxine according to Berg (*1*) is much simpler.

Procedure. From 0.1 to 0.2 gram of the fine powdered mineral is warmed 5 minutes with 5 to 10 ml. of 2 *N* hydrochloric acid, 50 ml. of water, and 0.5 to 1.0 gram of beryllium nitrate. Any residue is considered as gangue and eliminated by filtration. To the warm filtrate ammonia is added until a small precipitate remains, which is dissolved by dropwise addition of 2 *N* acetic acid. Now 10 to 20 ml. of 2 *N* ammonium acetate are added, followed by a 4% solution of oxine (8-quinolinol) in acetic acid, until no precipitation occurs, and heated to boiling. The aluminum oxine precipitate is filtered, after standing an hour, through a filtering crucible, washed with water, dried at 110° to 120° C., and weighed.

The results obtained by the analysis of pure cryolite from Greenland were:

Cryolite, G.	Al Oxinate, G.	Al, %	F Calcd., %
0.1255	0.2746	12.84	54.27
0.1248	0.2733	12.85	54.32
0.0768	0.1664	12.72	53.77
0.1088	0.2379	12.84	54.27
0.0947	0.2070	12.82	54.19
		Av. 12.81	54.16
		Theoretical 12.85	54.30

When an iron-containing cryolite is analyzed, ferric oxine together with aluminum oxine is precipitated, as shown by a greenish color of the precipitate instead of a yellow one. In this case the iron can be colorimetrically determined in a separate sample with thiocyanate after demasking with beryllium nitrate.

DETECTION OF PHOSPHATE IN PRESENCE OF LARGE AMOUNTS OF ALKALI FLUORIDE

Bucherer and Meier (*3*) as well as Neuhaus (*10*) have reported that the presence of fluorine ions prevents the complete precipitation of $(NH_4)_3PO_4.12MoO_3$. This interference is due to the formation of fluomolybdate ions, whereby a precipitation of phosphate ions by molybdate ions occurs only when all fluoride is transferred in fluomolybdate ions ($MoO_2F_4^{--}$). This must be kept in mind when phosphate is to be detected in the presence of fluoride by the molybdate reaction. The following data were obtained by heating (at 80° C.) the test solution with 5 ml. of ammonium molybdate solution (5 grams of salt in 100 ml. of water poured into 35 ml. of nitric acid, d, 1.2).

	P_2O_5, Mg	NaF, G.	
In 10 ml.	2	+ 0.0	Strong precipitation
	2	+ 0.08	Slow and incomplete precipitation
	2	+ 1.0	Yellow color on heating, nearly colorless on cooling; no precipitation
	2	+ 1.2	Slow yellow color on heating, colorless on cooling
	2	+ 1.5	No color, no precipitation

The above data show the danger that considerable amounts of phosphate may escape the sensitive molybdate test in a 1% sodium fluoride solution containing 0.02% phosphorus pentoxide. This danger is particularly great when smaller amounts of phosphate are to be detected in more concentrated alkali fluoride solutions. The masking action of fluoride, interfering with the phosphate test, can be eliminated by later addition of beryllium nitrate. Instead of using the nitric acid molybdate solution, a beryllium-containing molybdate solution can be used. The latter is especially indicated when phosphates are to be identified by the molybdate reaction through a spot test on filter paper (*6*).

DETECTION OF MOLYBDATE AND TUNGSTATE IN PRESENCE OF ALKALI FLUORIDE

The masking of reactions of molybdate and tungstate ions by fluorides (*4*) has probably found little consideration, because, in analyzing fluorine-containing material, all fluorine can generally be eliminated as hydrogen fluoride by fuming with concentrated sulfuric acid. This troublesome procedure where tungsten trioxide (and in part also molybdenum trioxide) remain together with insoluble sulfates and must be separated from the latter can be replaced by the simple demasking of fluorized molybdenic and tungstic acid. If, therefore, alkali molybdate and tungstate are to be detected in the presence of much alkali fluoride, an appropriate reagent for molybdate (or tungstate) is to be added to the test solution, followed by beryllium nitrate (solid or in concentrated solution). Different tests for molybdate and tungstate in fluorine-containing solutions were checked in this way. The limit of identification was found to be about one half that in fluorine-free solutions.

DETECTION OF BERYLLIUM IN MINERALS, ORES, AND ALLOYS

The specific colorimetric determination of beryllium described by Fischer (*9*) is based on the formation of sodium fluoberyllate and its reaction with alkaline quinalizarin solution, whereby the blue quinalizarin lake is formed. The formation of this lake and its stability toward hypobromite can be used for exact detection of beryllium in minerals, ores, and alloys. Silicatic or intermetallic bound beryllium can easily be transformed into potassium fluoberyllate by heating with potassium hydrogen bifluoride:

$$BeSiO_3 + 6HF \longrightarrow BeF_2 + 3H_2O + SiF_4$$

$$Be^\circ + 2HF \longrightarrow BeF_2 + H_2^\circ$$

$$BeF_2 + 2KF \longrightarrow K_2BeF_4$$

Experiments have shown that the products of fusing and sintering of potassium hydrogen fluoride with magnesium, calcium, ferric, aluminum, titanium, and zirconium oxides do not react with ammoniacal quinalizarin solution (50 mg. of quinalizarin in 100 ml. of 10% ammonia solution). It seems therefore that the following test is specific.

Procedure. A few milligrams of the material to be tested (powder or shavings) mixed with a threefold amount of potassium hydrogen fluoride are fused and sintered in a platinum spoon for 3 to 4 minutes. After cooling, the mass is treated with 2 ml. of cold water. After 5 minutes it is filtered or centrifuged. One drop of the clear supernatant liquid is placed on a spot plate and a drop of quinalizarin solution is added. In the neighboring depression of the spot plate a blank test is carried out with a drop of water. If beryllium is present, a blue color or blue precipitate, according to the amount of beryllium, is visible, whereas the ammoniacal quinalizarin solution remains unaltered—i.e., violet. If saturated bromine water is added dropwise, only the blank test is decolorized.

In this way, several beryllium-containing minerals were examined, among others a product of the following composition: 1% BeO, 25% Al_2O_3, 24% CaO, 3% MgO, 5% P_2O_5, 6% Fe_2O_3, 1% Mn_2O_3, 7% SiO_2. The test was always confirmative; the same was true when examining beryllium-containing alloys. By the above procedure not all beryllium (together with the other metals) is transformed into fluoride. By sintering with potassium hydrogen fluoride under the given conditions the fluorization occurs on the surface of the products examined, but because of the sensitive quinalizarin reaction the amount of potassium fluoberyllate formed is always sufficient to secure the specific detection of beryllium, even when present in small quantities.

DETECTION OF SMALL AMOUNTS OF IRON OR TITANIUM IN PRESENCE OF FLUORINE

Because of the decrease of ferric ion concentration when FeF_6^{---} ions are formed, detection of iron by the thiocyanate test fails in the presence of an excess of fluorine. The same is true of the delicate iron test with oxine in acetic acid solution. Therefore, when traces of iron are to be detected in alkali fluoride, iron(III) should be reduced with hydrogen sulfide to iron(II) and the latter detected through the color reaction with 1,1′-bipyridine (*8*). The demasking of FeF_6^{---} by beryllium chloride or beryllium sulfate permits identification of iron in fluorides, which might be the basis for a colorimetric determination.

Procedure. One drop of the test solution is mixed on a spot plate with some crystals of potassium thiocyanate, followed by addition of one drop of hydrochloric acid. If, after beryllium chloride or beryllium sulfate is added, a red or pink color is developed, iron is present.

In this way 2.5 micrograms of iron were detectable in presence of 25 mg. of ammonium fluoride.

Instead of the thiocyanate test, the more sensitive oxine test (green coloring or dark green precipitate) can be used. In this case a neutral test solution must be acidified with acetic acid, or an acetic acid solution buffered with alkali acetate, before an acetic acid solution of oxine is added.

Analogous to the behavior with iron, titanium ions are masked in the presence of an excess of fluorine, owing to the formation of TiF_6^{--} ions. Thus the well-known reaction for titanium in acid solution with hydrogen peroxide (formation of titanium peroxo compounds) is masked in the presence of fluorine. Here also by demasking through beryllium nitrate, titanium ions are produced, which form with hydrogen peroxide the yellow $TiO_2X_4^{--}$ ions (X = univalent acid radical). In the form of a spot test, the peroxide reaction for titanium has a limit of identification of 2 micrograms of titanium in one drop (0.05 ml.). The authors found that twice this amount can be detected in a saturated solution of alkali fluorides, when demasking with beryllium nitrate is used. This corresponds in the case of ammonium fluoride to a ratio of 1 part of titanium to 6100 parts of ammonium fluoride.

ACKNOWLEDGMENT

The authors wish to thank L. Baumfeld for carrying out many tests for detection of beryllium in minerals, ores, and alloys.

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Microdetermination of Free Formaldehyde

MORRIS TANENBAUM AND CLARK E. BRICKER, *Princeton University, Princeton, N. J.*

The available methods for the determination of small amounts of free formaldehyde in the presence of considerable quantities of various other organic compounds are either time-consuming or lack accuracy, specificity, and/or sensitivity. This investigation was initiated to rectify this situation. A sensitive, reproducible, and rapid spectrophotometric procedure has been developed for the determination of free formaldehyde in the presence of combined formaldehyde (formals) and of many organic compounds. Since the procedure used involves the time-sensitive reaction of formaldehyde with phenylhydrazine and potassium ferricyanide, the optimum conditions have been established for the development and stabilization of the characteristic red color. The procedure for determining free formaldehyde should be applicable to nearly all analytical problems where it is necessary to determine traces of uncombined formaldehyde. This method should be particularly useful to the plastic industry, in toxicological work, and in various phases of biochemical and bacteriological research. Furthermore, it affords a simple means of following reactions which utilize formaldehyde.

ALTHOUGH many gravimetric methods for the quantitative determination of formaldehyde have been successfully developed and used, the attempts at colorimetric estimation of formaldehyde solutions have been limited to the Denigès method employing Schiff's fuchsin bisulfite reagent (*1*, *4*, *7*) and to the more recent chromotropic acid method, suggested by Eegriwe (*6*), adapted as a quantitative procedure by Boyd and Logan (*2*), and later improved by Bricker and Johnson (*3*). The chromotropic acid method is sensitive not only to free formaldehyde, but also to any substance which will yield formaldehyde upon hydrolysis in concentrated sulfuric acid. However, the only method previously available for the colorimetric determination of free formaldehyde in the presence of combined formaldehyde (in the form of acetals, etc.) was the procedure employing the Schiff reagent (*1*) which required the daily preparation of fresh standards and also a standing period of 6 hours prior to reading in the colorimeter. It was the obvious need for a more rapid and satisfactory method for the determination of free formaldehyde in the presence of combined formaldehyde that prompted the present investigation.

Pittarelli (*9*) found that a solution of acetaldehyde when treated with phenylhydrazine hydrochloride and then with a mixture of sulfanilic acid and sodium nitrite solutions, followed by a few milliliters of 25% sodium hydroxide, gave a crimson coloration. Formaldehyde apparently did not react similarly. Penzoldt and Fischer (*8*) reported on a variation of this reaction by using diazobenzenesulfonic acid and sodium amalgam as the reagents and found that several aldehydes gave the crimson coloration.

In a preliminary study using solutions of phenylhydrazine hydrochloride, diazobenzenesulfonic acid, and sodium hydroxide as reagents, the crimson coloration could be obtained from several aliphatic aldehydes whereas ketones did not give the characteristic color and aromatic aldehydes reacted only slowly. Because the test was extremely sensitive for formaldehyde and the color formed by formaldehyde was less affected by moderate manipulation of the variables than the color produced by higher aldehydes, the authors suspected that further investigation might yield a sensitive, yet rapid, method for the quantitative estimation of microquantities of free formaldehyde. Although the method showed great promise, there were fluctuations in the reproducibility that no reasonable amount of control of the several variables could remedy. These variations were finally traced directly to the diazobenzene sulfonic acid reagent. This solution undergoes decomposition so rapidly that even under the most favorable conditions its potency began to diminish 2 to 3 days after its preparation and the solution was completely useless after a week. The solid diazo compound was more stable, but within 1 to 2 months after its preparation it also exhibited signs of decomposition. Furthermore, the necessity of close control of the time variables during the course of reaction, solubilities, and other factors prohibited the use of the diazonium salt in its solid form.

During the preliminary work, the color was greatly intensified if an attempt was made to determine formaldehyde in absolute methanol. This action was finally traced by the chromotropic acid procedure to an oxidation of the methanol to formaldehyde by the diazonium salt. (This reaction might well be used for the determination of small amounts of certain diazonium compounds.) The knowledge of the oxidizing power of the diazonium reagent, plus the fact that evolution of a gas was noted immediately upon addition of the diazonium reagent and not upon addition of the sodium hydroxide, led to the interesting possibility that the color might be due to an oxidation by this reagent, rather than to any sort of coupling which one might automatically connect with a color produced by a diazonium compound.

Solutions of standard oxidizing agents were then tried in place of the diazonium solution. With a ferric sulfate solution, upon long standing after the addition of the sodium hydroxide, a red color, visually identical to the color produced by the diazonium reagent, was formed. The results of the various oxidizing agents that were tried are tabulated in Table I.

Table I. Effect of Various Oxidants

Oxidant	Immediate Observation	After 1 Hour
Ferric sulfate	No color	Deep red
Potassium ferricyanide	Deep red	Deep red
Silver nitrate	No color	No color
Potassium iodate	Pale red	Red-orange
Potassium bromate	Pale red	Red-orange
Potassium permanganate	Pale red	Red-orange

Potassium ferricyanide showed the greatest promise. A spectrophotometric comparison of the color produced by ferricyanide with that produced by the diazonium salt showed the two colors to be identical, as demonstrated in Figure 1.

The similarity of reagents recalled to mind a procedure for formaldehyde suggested by Schryver (*11*) and later by Desnuelle and Naudet (*5*), and thoroughly investigated in this laboratory by Roberts (*10*). The previous workers suggested a reaction with phenylhydrazine hydrochloride, potassium ferricyanide, and concentrated hydrochloric acid. The work of Roberts showed that this color, which also had a characteristic absorption peak at 520 mμ, was much too unstable for accurate work. A spectrophotometric analysis of the color developed in an acid medium showed it to be very similar to the color developed under basic conditions with the exception of a slight absorption shoulder at 545 mμ. A similar experiment utilizing phenylhydrazine hydrochloride, *p*-diazobenzenesulfonic acid, and concentrated hydrochloric acid also gave a color spectrophotometri-

cally identical to the Desnuelle and Naudet color. Furthermore, when the color was formed according to the acid procedure, and when the solution was made basic to litmus with sodium hydroxide, the absorption band at 545 mμ disappeared; the color was spectrophotometrically identical to the color developed by the basic procedure using either *p*-diazobenzenesulfonic acid or potassium ferricyanide. These spectra are shown in Figure 1.

Although the procedure described in this paper is not entirely new, it is a variation which does enable the use of a very sensitive color reaction for the determination of formaldehyde which has not heretofore proved completely satisfactory.

EXPERIMENTAL

Reagents. Phenylhydrazine hydrochloride, 7.5% aqueous solution. Dissolve 3.75 grams of reagent grade solid in warm water and filter off any insoluble material.

Potassium ferricyanide, 5% aqueous solution

Sodium hydroxide, 10% aqueous solution

Isopropyl alcohol, reagent grade

Procedure. The sample to be analyzed should not contain over 15 micrograms of formaldehyde and should not be over 0.6 ml. in volume. Sufficient isopropyl alcohol and water are added to give a total of 1.0 ml. with an isopropyl alcohol content of 40 to 50%. If solid, a weight sufficient to give a formaldehyde concentration of 0 to 15 micrograms is dissolved in 1.0 ml. of 50% isopropyl alcohol. The samples are conveniently handled in 18 × 150 mm. test tubes.

To the sample thus prepared, 0.5 ml. of phenylhydrazine hydrochloride solution is added and the solution is allowed to stand 10 ± 1 minute. Then 0.3 ml. of potassium ferricyanide solution is added and after 5 ± 0.5 minute, 2.0 ml. of the sodium hydroxide solution are added. After 4 ± 1 minute the solutions are diluted to approximately 20 ml. in the test tubes, then transferred to 25-ml. volumetric flasks, and diluted to volume. The optical densities of the solutions are then measured at 520 mμ against a reagent blank 10 ± 3 minutes after the initial dilution to 20 ml. The amount of formaldehyde is then found by comparison with a suitably prepared calibration curve.

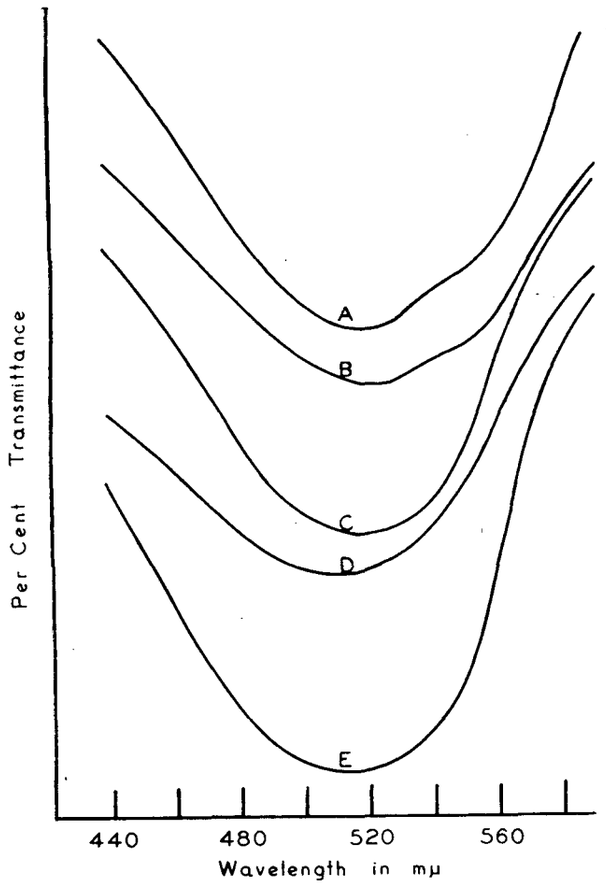

Figure 1. Photometric Spectra of Characteristic Formaldehyde Colors

A. Diazonium method with acid development
B. Ferricyanide method with acid development
C. Diazonium method with basic development
D. Ferricyanide method with acid development followed by neutralization
E. Recommended ferricyanide method with basic development

DEVELOPMENT OF METHOD

The dependence of the color development upon the variables of reagent concentration and standing time between addition of reagents is striking. A systematic investigation of these variables has been made.

Table II. Reaction Time of Ferricyanide

Time between Addition of $K_3Fe(CN)_6$ and NaOH, Min.	Extinction
1	0.535
3	0.548
5	0.560
7	0.550
12	0.532
16	0.503
20	0.463

The amounts of the various reagents used are limited by their solubility and by the requirement that a sufficient concentration be present so that the amount of these reagents reacting with the formaldehyde will be negligible compared with the total concentration. As in all colorimetric work, this consideration is necessary in order to ensure the validity of the blank.

The upper limit of phenylhydrazine hydrochloride that can be added is determined by the solubility of this reagent and its reaction products. The isopropyl alcohol–water solvent is employed to overcome this difficulty. However, even in this solvent, use of reagents much in excess of the recommended amounts will result in the formation of a precipitate. On the other hand, if either the amount of isopropyl alcohol or the strength of the sodium hydroxide solution is much increased, then the isopropyl alcohol tends to salt out upon the addition of the sodium hydroxide. This results in an immiscible layer which extracts a great deal of the color. Although this immiscible layer disappears upon the dilution to 25 ml., experience shows that its presence is sufficient to make reproducibility very difficult.

All of the following investigations were performed on 1-ml. samples containing approximately 10 micrograms of formaldehyde and 40% isopropyl alcohol. Extinctions were read at 520 mμ against a reagent blank unless otherwise specified.

The effect of the phenylhydrazine hydrochloride concentration was studied by employing 0.2, 0.4, 0.6, 0.8, and 1.0-ml. portions, respectively, of the 7.5% reagent and otherwise following the recommended procedure. The extinctions observed were 0.383, 0:411, 0.414, 0.393, and 0.384, respectively. Since the color produced by 0.4- and 0.6-ml. portions of this reagent was practically identical and showed maximum sensitivity, the optimum concentration of this reagent was taken at 0.5 ml.

The effect of the time interval between the addition of the phenylhydrazine hydrochloride and the potassium ferricyanide was studied. Using the recommended amount of the phenylhydrazine reagent, the potassium ferricyanide was added after 5, 10, 20, and 30 minutes, respectively. Since 10 minutes produced nearly the maximum extinction with little (less than 2%) change at 20 or 30 minutes, the procedure time was set at 10 minutes.

The extinction goes through a maximum as both the concentration of phenylhydrazine and its reaction time are increased. It would seem that the color producing agent must, then, be an intermediate in the formation of the phenylhydrazone.

The potassium ferricyanide concentration was also studied by employing 0.1-, 0.2-, 0.3-, 0.4-, and 0.5-ml. portions of the 5% reagent and again following the recommended procedure. The

Table III. Reaction Time of Sodium Hydroxide

Time Elapsed between NaOH Addition and Dilution, Min.	Extinction
2	0.569
3	0.573
4	0.574
5	0.575
8	0.580
10	0.595
15	0.563
20	0.550
30	0.548

Table IV. Stability of Color

Time after Dilution, Min.	Extinction *vs.* H_2O		
	Blank	Sample	Sample blank
6.5	0.056	0.512	0.456
10	0.057	0.511	0.454
15	0.058	0.510	0.452
20	0.060	0.504	0.444
25	0.062	0.501	0.439
30	0.064	0.498	0.434
40	0.070	0.492	0.422
90	0.134	0.511	0.377

Table V. Interferences

Interfering Substance	Maximum Tolerance Mg./Ml.,	Type Interference
Methanol	50.0	+
Ethanol	400.0	+
Acetaldehyde	0.05	+
Isobutyraldehyde	0.20	+
Benzaldehyde	0.20	−
Acetone	2.5	−
Ethylamine hydrochloride	20.0	−
Propylamine	2.0	−
Phenol	50.0	−
Formic acid	10.0	−
Pentaerythritol diformal	>50.0	?
Pentaerythritol	>50.0	?

measured extinctions were 0.396, 0.485, 0.547, 0.543, and 0.546, respectively, and clearly show that the volume of this reagent is not critical above 0.3 ml. However, because the larger amounts gave an apparently undesirable precipitate, 0.3 ml. of the potassium ferricyanide solution is recommended.

The effect of the variation of the time between the addition of the potassium ferricyanide and the addition of the sodium hydroxide was studied and these results are given in Table II.

From these results it is evident that the optimum reaction time for the potassium ferricyanide is 5 minutes. This time should be more closely regulated than the other reaction times, because the color development goes through a comparatively sharp maximum. Thus a variation of more than ±30 seconds is to be avoided.

The variation with respect to sodium hydroxide concentration was investigated by using 2-ml. volumes of 10, 20, 30, and 40% sodium hydroxide. Although these extinctions varied only between 0.610 and 0.628 and would indicate that the amount of sodium hydroxide was not critical, the higher concentrations of this reagent caused the formation of a second layer. Thus, 10% sodium hydroxide was chosen as the optimum concentration.

Using the recommended amounts of reagents, the time elapsed before dilution was studied and the results are given in Table III.

The time of 4 minutes was chosen because the change in extinction with time is slow at this point.

The isopropyl alcohol content of the sample is the final reagent variable; the sample remains clear throughout the determination only when it is at least 40% isopropyl alcohol. Although there is no significant difference between the extinction of samples with 10 to 50% isopropyl alcohol, it is desirable to keep the sample free from any precipitate. Above 60% isopropyl alcohol, there is a pronounced decrease in the extinction for a given amount of formaldehyde. This is possibly due to an oxidation of appreciable amounts of the alcohol to acetone which interferes negatively.

The extinction of a blank solution and of a solution containing 10 micrograms of formaldehyde was measured at various times after the initial dilution to 20 ml. These results, as well as the difference between the two extinctions, are given in Table IV.

The steady increase in extinction of the blank (and of the sample at 90 minutes) is due to the fact that the solution slowly becomes cloudy. Although satisfactory readings can be obtained at any time up to 15 minutes after the initial dilution, a time of 10 minutes was chosen because the color changes only slowly at this point. Furthermore, this also allows sufficient time to dilute several samples accurately to 25 ml. and to make the necessary readings.

It is obvious that the color is not completely stable with time. There is, however, a very decided improvement over the stability of the color as formed under acid conditions where the decrease is approximately 1% per minute.

STABILITY OF REAGENTS

Although the potassium ferricyanide solution changes color on standing, it seems to retain its full potency indefinitely. The phenylhydrazine hydrochloride solution slowly darkens and a brown sediment appears after about a week. After filtration, this reagent continues to give reproducible results. However, because of the change in color of the reagents, the extinction of the samples must always be measured against a reagent blank.

INTERFERENCES

The effect of various compounds upon the formation of the characteristic color has been investigated. Various weights of the interfering substances plus known amounts of formaldehyde were analyzed according to the recommended procedure. The results are given in Table V. The values under the column labeled maximum tolerance indicate the quantity of the given substance which will produce no error in the formaldehyde determination. Larger amounts will produce the type of interference given in the last column.

The positive interference from acetaldehyde and isobutyraldehyde is due to the nonspecificity of the reagents toward aliphatic aldehydes. However, as can be seen, the sensitivity decreases as the chain length increases. The only other cases of positive interferences are methanol and ethanol. This undoubtedly arises from the production of formaldehyde and acetaldehyde by oxidation of the respective alcohols. Similarly, we could expect a positive interference from any other substance which will, upon potassium ferricyanide oxidation, yield microamounts of formaldehyde or larger amounts of other aliphatic aldehydes.

The negative interference of acetone and benzaldehyde is believed to be due to the consumption of the phenylhydrazine, thus decreasing the effective concentration for action on the formaldehyde. As for the interference of amines, it is evident from Table V that the main interference is due to the basic function of the amine, because the hydrochlorides can be tolerated in amounts approximately 10 times as great. Also, the pH change explains the interference of phenol and formic acid. It is possible that careful buffer control might raise the tolerance of these substances immensely. This has not been investigated.

The above interferences refer only to the quantitative determination of formaldehyde. Qualitatively it is possible to detect microamounts of formaldehyde in the presence of immense amounts of substances such as benzaldehyde, acetone, and other negatively interfering substances. The only compounds that will completely prevent formation of the characteristic color are basic substances. This is in accordance with the suggestion that the color depends upon the formation of a hydrazone intermediate. Substances giving positive interferences will interfere in the qualitative detection of formaldehyde.

APPLICATIONS

Inasmuch as the recommended procedure is very sensitive (0.055 extinction unit per microgram of formaldehyde), and the color developed shows linearity with the formaldehyde concentra-

tion up to at least 15 micrograms this method is one of the most sensitive and useful systems for determining formaldehyde. Furthermore, although the time required for a single analysis is about 30 minutes, it is very simple to run four or six determinations concurrently in the same amount of time. The accuracy of the method is well within 2%.

The advantage of the proposed method for determining formaldehyde is that the reaction is run in essentially neutral and basic mediums. Since compounds that may hydrolyze to yield formaldehyde under acid conditions do not interfere, it should afford a very rapid and sensitive means of determining free formaldehyde in such materials as phenolic resins, formals, and trioxane. This method, combined with the chromotropic acid procedure, has been used to good advantage in a preliminary investigation of the specific rate constant in the formation of pentaerythritol monoformal.

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Magnetically Controlled Quartz Fiber Microbalance

FRANK C. EDWARDS AND ROBERT R. BALDWIN[1]
Institute for Atomic Research, Iowa State College, Ames, Iowa

The design, simplified techniques of construction, and uses of an electromagnetically controlled quartz fiber microbalance are described. The balance is capable of weighing directly an unbalanced load as large as 1.2 mg., carrying a counterbalanced load of 100 to 150 mg., and giving a sensitivity of 0.1 microgram per 6 minutes of arc. The one-piece all-quartz balance unit fits into a 25-ml. diameter tube 12 cm. long. The balance may be used under wide conditions of temperature, pressure, and reagents in completely closed systems. The balance is controlled by an external variable electromagnetic field.

LABORATORY microbalances, commercially available, are limited in sensitivity to 1 microgram. A temperature difference of as little as 0.1° C. has been reported by Lindner (*7*) to introduce a weighing error of several micrograms, in the case of a combustion boat and glass pig (total weight of 4 grams). A change in barometric pressure of a few millimeters will introduce errors due to change in buoyancy. A change in the humidity gives rise to significant variations in the rest point of a commercial microbalance, for several reasons discussed by Corwin (*2*).

Quartz fiber microbalances are not as susceptible to temperature changes, because of the low coefficient of expansion of quartz. The effects due to changing barometric pressure and humidity may be eliminated by operating such a balance in a vacuum. The idea of a microbalance constructed of quartz fibers is not a new one; the idea of electromagnetic control goes back to Ångström in 1895 (*1*, *3*). Reviews of the developments in these fields have been published by Emich (*3*) and Gorbach (*4*).

Most significant to the development of the balance described in this paper was the work of Steele and Grant (*9*), who first constructed a complete balance, of the central knife-edge type, from fused quartz. Also of significance was the work of Petterson (*8*), who improved the design and gave a detailed description of the theory of such instruments. Emich (*3*), Stock (*10*, *11*), and Wiesenberger (*13*) used Ångström's idea of electromagnetic control in their investigations. The authors are particularly indebted to Johns (*5*), who constructed several balances of a design very similar to the one described in this paper. He used wax or selenium to seal the central axis fiber and pan suspension bows to the beam.

An electromagnetically compensated quartz fiber balance is now more practical and versatile because of the use of improved magnet materials and balance designs. The assembly of the balance described is simplified through the use of unique construction techniques. This balance may be used for conventional microanalytical uses, but its compact design with external control makes it particularly useful as a research tool.

[1] Present address, Central Laboratories, General Foods Corp., Hoboken, N. J.

DESIGN OF BALANCE

The balance described is an equal-arm lever balance in which the beam is rigidly fastened to a flexible supporting axis fiber. It is not a true torsion balance, for the force of gravity is only slightly compensated by the torsion of the support fiber. Magnetic compensation is employed to oppose the force of gravity. Every effort has been made to design and construct the balance in such a manner as to combine the features of a high sensitivity quartz fiber microbalance and magnetic compensation, which allows greater load capacity and range of weighings as well as external control.

Important Parts of Microbalance. A clearer idea of the principles and terminology involved may be obtained by reference to the perspective drawing in Figure 1.

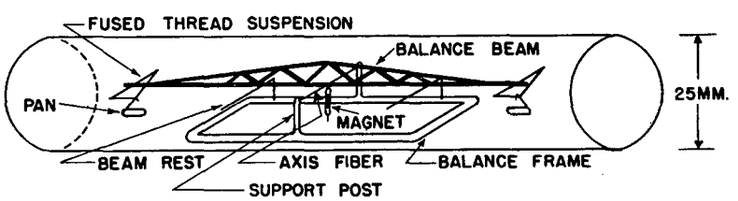

Figure 1. Balance Unit

BEAM FORM. The beam of the balance is of a braced simple bridge truss design and is constructed of small quartz rods. A lightweight beam of maximum strength is possible using this design.

CENTRAL AXIS. The central axis fiber suspends the beam between support posts on a rectangular

quartz framework. This axis fiber is fused to the posts of the framework and to the beam. No seals except quartz fusions are used, so that a complete balance unit is an integral piece of quartz. Balances may be constructed by sealing the suspension fibers to the beam and the framework with selenium or wax; however, such balances are less reproducible over periods of time and are much more affected by temperature changes and chemical reactions than are all-quartz balances.

TERMINAL AXES AND FUSED THREAD SUSPENSIONS. The terminal axes are placed equidistant from and parallel to the central axis fiber. Quartz thread suspensions are fused to the terminal axes. These bow-shaped suspensions are used to support the pans of the balance. They must be in the same plane as, or very slightly below, the central axis fiber.

BEAM MAGNET. A small permanent bar magnet of Alnico or Cunife is mounted below the midpoint of the balance beam and perpendicular to its length. This magnet is sealed in a quartz capillary which in turn is fused to the beam. Use of the newer magnet materials, with their high ratio of magnet strength to weight, make a lightweight beam unit possible and magnetic control more practical. Portions of steel needles may be used, but they have a greater tendency to lose their magnetism, thus making numerous calibrations necessary.

QUARTZ ROD FRAME AND BEAM RESTS. The quartz rod frame is illustrated in Figures 1 and 2. The centrally located posts are used to support the central axis fiber. Beam rests are positioned to limit the swing of the beam.

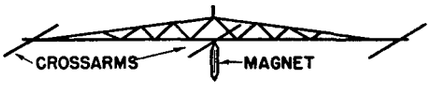

Figure 2. Quartz Balance Frame

EXTERNAL CASE OF BALANCE UNIT. A borosilicate glass tube 25 mm. in diameter and 12 cm. long (see Figure 1) encases the entire balance assembly. This compact unit may be inserted into closed systems for use in a variety of research and analytical problems.

PANS. The design of pans is determined by the particular problem for which the balance is to be used. Pans are conveniently shaped from platinum foil and welded to platinum wire hooks. Pans of split mica, suspended on quartz fiber hooks, have also proved satisfactory in some instances.

Operating Principles. MAGNETIC COMPENSATION FOR WEIGHING. Ångström's method of magnetic compensation was briefly this (*1*):

A small permanent bar magnet was attached to the balance beam, below and parallel to the length of the beam. The orientation of this magnet and hence the weight lifted by the balance were controlled by a variable electromagnetic field which was perpendicular to the magnet. This field was produced by current flowing in a solenoid, the end of which was below (or above) the balance magnet. The current passing through the coil was measured with a sensitive galvanometer.

A second method of orienting the permanent magnet and the regulating solenoid was chosen for the balance described in this paper.

The permanent magnet is mounted below the midpoint of the beam and perpendicular to its length. Because the field strength, H, within a long solenoid equals $4\pi nI/10$ where n is equal to the number of turns per centimeter of length of the solenoid and I is the current in amperes, it is apparent that the magnetic field acting on the permanent magnet of the balance is affected only by changes of the current in the solenoid. Furthermore, the moment of force, tending to produce rotation on a bar magnet placed at right angles to the lines of a magnetic field, is the product of the strength of the field and the magnetic moment of the magnet. The magnetic moment of a permanent magnet is the product of the pole strength and the distance between the poles; for Alnico or Cunife magnets this quantity is constant. Hence, the rotation of the magnet and correspondingly the weight lifted by the balance are determined by, and are directly proportional to, the current passing through the solenoid. A measure of this current is obtained by placing a fixed resistance in series with the solenoid. The voltage drop over this resistance, and hence the current through the solenoid, are measured with high precision using a potentiometer.

If the strength of the permanent magnet is not known, an empirical adjustment of the current, the number of coil turns, and of the type of potentiometer needed for any setup must be made.

The use of magnetic compensation has a further advantage. The long period oscillations, so disconcerting in a commercial microbalance, or in a torsion balance of the same sensitivity, are eliminated by a magnetic damping effect which permits immediate adjustment of the balance.

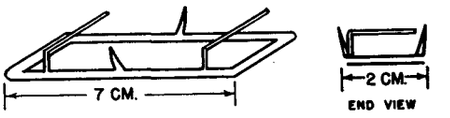

Figure 3. Beam with Cross Arms and Magnet Ready for Mounting on Frame

ELECTRICAL REGULATING SYSTEM. The solenoid surrounding the balance consists of 1000 turns of No. 26 cotton-covered copper magnet wire. To control the current in the solenoid, and hence the magnetic field at its center, three variable resistances (2500 ohms, 25 ohms, 1 ohm) are placed in series in the circuit. Direct current is supplied to the circuit from one or more storage batteries. To measure the current, a fixed 3-ohm resistance of No. 32 Copel wire, which has a negligible temperature coefficient of resistance, is placed in series in the circuit. The IR drop over this resistance is carefully measured with a Leeds and Northrup Type K potentiometer. Refinements which may be incorporated in the electrical system include a reversing switch, to reverse the current flow in the solenoid, and a milliammeter, to aid in the adjustment of the potentiometer. A selector switch may be used to connect several solenoids successively so that several balances may be controlled with one electrical regulating system.

ARRESTMENT SYSTEM. The balance is used as a null instrument and so beam rests are placed to limit the swing of the beam (Figures 1 and 2). This feature of the design is useful for the following reasons. A very compact design may thereby be attained. The effective arm length of the balance is more likely to remain constant. Danger of damaging the balance through large accidental swings of the beam is practically eliminated, and there is decreased danger of tipping the pans and spilling a sample.

ADJUSTMENT OF SENSITIVITY. A stable, sensitive balance must have its center of gravity on, or very slightly below, its central axis fiber. Although during the construction of this balance every effort is made to maintain a weight symmetry, a final adjustment is usually necessary. This is accomplished by fusing bits of quartz to the post at the top of the beam, or to the capillary below the magnet, until the balance is stable and as sensitive as is required.

READING SYSTEM. The swing of the beam is observed with a reading microscope.

The microscope fitted with a cross hair is focused on a pointer fiber drawn from one end of the beam. With the pan empty or tared, current is supplied to the coil until the balance swings free of the beam rests. The pointer fiber is made to coincide with the cross hair in the field of the microscope. This position is used as a zero point position, and then as the equilibrium position for any subsequent weighing. The microscope must remain fixed with respect to the balance during a series of weighings. The fixed positions of the pointer fiber, when the balance is riding the beam rests, are in the field of the microscope, and thus provide a useful check on the relative orientation of microscope and balance.

CONSTRUCTION OF BALANCE

Drawing and Fusing Quartz Fibers. This paper covers only the design and particular construction techniques essential to the preparation of the balance described. Further information on quartz working is given by Strong (*12*).

With the exception of a micro oxygen torch, little special equipment is necessary. It is important to use a sensitive valve system to regulate the gas and oxygen flow. The micro torch tip may be

made from a short length of quartz tubing, shaped to give a flame 1 to 2 mm. long.

The quartz fibers from which the balance is constructed are drawn from quartz rods 4 to 8 mm. in diameter. One may draw a fiber of size from 10 microns to 1 mm. in diameter using an ordinary gas-oxygen laboratory hand torch. Bends in quartz fibers may be made by holding one end of the fiber on a graphite plate with a small weight, heating a portion of the fiber with the micro flame, and then bending the fiber by means of tweezers. When the micro burner flame is brought in contact with the graphite plate, water condenses and prevents heating to the temperature necessary for bending quartz. This disturbing effect is eliminated by placing the graphite block on a hot plate, and making bends and seals either just over the edge of the block, or over a hole drilled in the surface of the graphite. Quartz fiber construction is easier if the fibers involved are held firmly in position with small weights before the fusing operations are attempted. Quartz fibers are cut to the proper size by using a notched pair of scissors or a tungsten carbide pencil to scratch the fiber before breaking it.

Making Quartz Frame and Beam. The quartz frame upon which the balance is mounted (Figure 2) is made from 3-mm. quartz rod. The support posts for the axis fiber and the beam rests are made from 1-mm. rod.

The beam is constructed of quartz fibers and braced as shown in Figure 3, in order to keep the beam as light as possible and yet rigid enough to support a total dead weight of 300 to 350 mg. The fibers used for the beam construction are approximately 0.3 mm. in diameter and weigh 1.5 to 2 mg. per cm. of length.

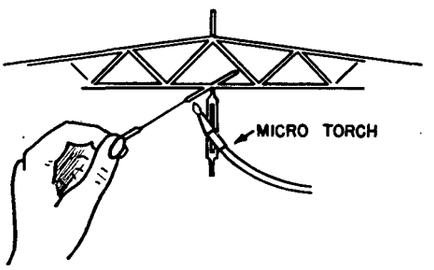

Figure 4. Pulling Axis Fiber from Central Cross Arm

Attaching Beam Magnet. The Cunife, or Alnico, magnet sealed in a thin quartz capillary is fused to the beam as in Figure 3. This magnet weighs about 10 mg., is 5 to 7 mm. long, and has a diameter of about 0.6 mm. Care in selecting a capillary of the proper size, and in sealing the magnet in this capillary, is necessary to eliminate all play. The position of the magnet below the beam is fixed to counterbalance the weight of the quartz bracing and upper span. This places the center of gravity of the beam and magnet unit close to the central axis fiber.

Attaching the Three Axes. The three cross arms, corresponding to the three knife-edges on an ordinary analytical balance, and consisting of quartz fibers weighing about 1 mg. per cm., are fused to the beam, preparatory to the mounting of the central suspension fiber and the terminal axes suspension fibers (see Figure 3). Because in a sensitive balance it is essential that these cross arms lie in the same plane, and be mutually parallel, the fusing operation is carried out on a flat block of graphite to ensure proper alignment.

Mounting the Beam. During the next operations bits of sealing wax or selenium are used to hold the balance tight to the rests, which are bent upward to hold the beam in an approximation to its final position.

Using a carefully adjusted micro flame, quartz threads of the order of 10 microns in diameter and about 1 cm. long are pulled from the central cross arm as illustrated in Figure 4. The fusion of these suspension fibers to the support posts of the frame must be carried out with caution to prevent melting them in two (see Figure 5). If properly completed, the central axis fiber is securely fused to the post. This point of fusion is then carefully heated until the surface tension of the molten ball of quartz pulls the slack in the fine suspension fiber into the post. This same method is used in the mounting of the terminal axes suspension fibers.

In using these techniques, fusions directly involving the delicate suspension threads are not required. This makes the construction considerably easier than if the quartz threads were drawn in a separate operation and then fused to the larger fibers.

Unless the central suspension fiber is under considerable tension, anomalous effects will be observed during operation of the balance. In order to tighten the central suspension fiber, and so remove all slack, the following technique is employed.

The frame and balance are oriented with the central axis fiber in a vertical position. The balance beam is freed from the beam rests for these operations. As illustrated in Figure 6, a hook of quartz fiber is fused to the lower post and a 1- to 2-gram weight is suspended. The necessary tension is provided by heating the post to the softening point, allowing the weight to pull down. The beam rests are now lowered to allow the beam to rock about 1 mm. Because the swing of the beam is to be observed by a reading microscope, a fine fiber is drawn from a beam end, parallel to the length of the beam, to serve as a pointer.

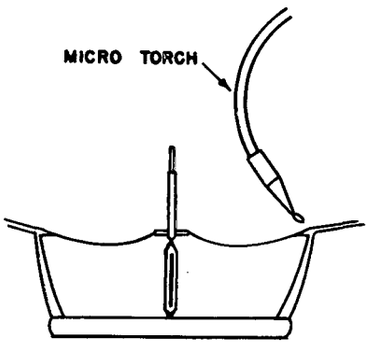

Figure 5. Shrinking Axis Fiber

Magnetization. The permanent magnet may be magnetized following completion of the above construction. The entire balance assembly is placed within a borosilicate glass tube 25 mm. in diameter and about 12 cm. long. This unit is placed in a magnetic field created by a magnetizing force of at least 4000 ampere-turns per inch of the Alnico or Cunife permanent magnet material. By magnetizing the permanent magnet as a final step in the balance construction, possible demagnetization resulting from heating during the fusing steps is counteracted.

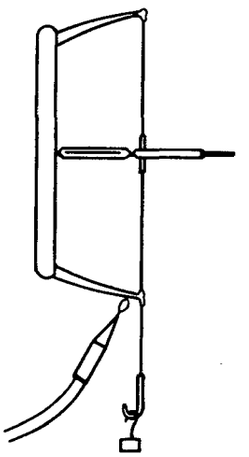

Figure 6. Final Tightening of Axis Fiber

OPERATION OF THE BALANCE

Making a Weighing. The operation of the balance is simple.

With the pan empty or tared, current is supplied to the coil until the balance is brought to a zero point, using a reading microscope fitted with a cross hair to focus on the pointer fiber. The voltage drop across the standard resistance is measured. Any change in weight will require a second adjustment of current, and thus a second voltage reading. The difference between these readings is directly proportional to the regulating current, which in turn is directly proportional to the change in weight.

Calibration of Balance. The instrument is calibrated by adding to the pan known standard weights, such as sections of a weighed length of quartz fiber or fine platinum wire. A known amount of a suitable salt—e.g., potassium chloride solution evaporated to dryness—may also be used. The method and problems of calibration by these methods have been described by Kirk *et al.* (*6*).

For illustrating the sensitivity of the balance and the linearity of the relation, weight *vs.* voltage, calibration data are listed in Table I and illustrated in Figure 7.

Table I. Calibration Data

Weight Units	Eyepiece Scale Position	Potentiometer Reading, Volt	Sensitivity, Volt per Division	Eyepiece Scale Zero Position, Volt	Weight, Micrograms
0	+4.4 −7.4	0.14456 0.14268	0.00016	0.14386	0
1	+3.5 −6.9	0.07095 0.06936	0.00015	0.07039	87
2[a]	−7.1 +1.7	0.01635 0.01499	0.00014	0.01526	174
3	−10.9 +4.7	0.09698 0.09431	0.00017	0.09505	261
4	−2.5	0.16520	...[b]	0.16480	348
5	−12.1 +3.3	0.24317 0.24075	0.00016	0.24128	435

[a] Direction of current in regulating coil reversed.
[b] No data obtained.

The standard weights used in this calibration were sections of a quartz fiber. No special pains were taken in the preparation of these weights. A length of quartz fiber was weighed and measured, giving a factor of 0.087 mg. per cm. of length. One-centimeter sections were cut with a razor blade without magnification. As a result the average agreement of the unit weights is of the order of 5%. The balance pans were initially tared so the right pan was lighter. Upon addition of the second weight unit, the right pan became heavier and a reversal of the direction of the coil current was necessary.

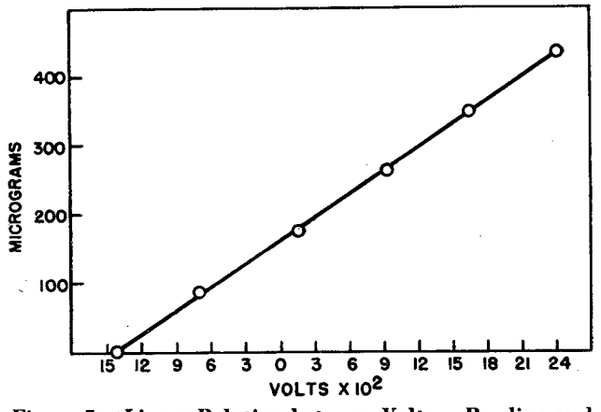

Figure 7. Linear Relation between Voltage Reading and Weight Determined

Figure 7 clearly represents the linear relation between the voltage reading and the weight determined.

Operating Precautions. As with any microbalance, the system should be protected from mechanical shock, and the usual precautions should be taken against uneven lighting and external electrostatic or magnetic influence.

PHYSICAL CHARACTERISTICS OF BALANCE

Sensitivity and Reproducibility. The sensitivity of balances of this type can be most exactly defined in terms of the weight required to give a deflection of the balance beam through a definite arc. In this case 6 minutes of arc have been chosen, which corresponds to a displacement of one division on the eyepiece scale.

In column 4 of Table I the voltage equivalent of this displacement is tabulated over the weight range 0 to 435 micrograms and is seen to be constant over this range. The sensitivity of this particular balance is thus about 0.2 microgram per 6 minutes of arc.

Reproducibility as used here is a function of the particular operation for which the balance is used. If a reaction is carried out without removal of the pans, the balance will "reproduce" readings to the same limit as the sensitivity of the balance. However, if frequent manipulation of the pans is necessary during the experiment, the reproducibility or precision of a weighing may not be the same as the sensitivity. To illustrate what can be done, a pan was removed from the balance, placed on a platinum foil, and then returned to position on the suspension bow. In a series of eleven measurements the balance reproduced the original reading within the sensitivity limit. However, more drastic manipulations which involve heating the pans may change the reproducibility as much as a few micrograms.

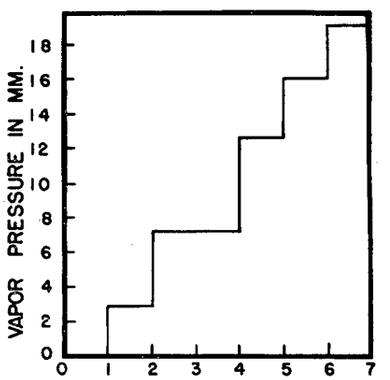

Figure 8. Waters of Hydration of Magnesium Sulfate

Load Capacity and Weighing Range. The load capacity of the microbalance—the counterbalanced weight of a sample and pan—may be as much as 150 mg.

The balance will weigh directly, using the magnetic control method, an unbalanced weight difference as large as 1.0 to 1.2 mg., if advantage is taken of the reversing of the coil current in the manner illustrated in the calibration procedure (see Figure 7).

Behavior under Damping. Adjustments and readings may be made in a few seconds, because of the magnetic damping effect and the use of the balance as a null instrument.

Temperature Range. The temperature range of the balance is considerable. Through the use of the special Alnico or Cunife magnet materials, the balance unit itself may be used up to 150° to 200° C. At these temperatures the permanent magnet loses some of its residual magnetism. Single pan reactions may be carried out at extremes of temperature if the pan is suspended in a trap, thus protecting the permanent magnet.

Stability. The balance is able to withstand reasonable use and is mechanically stable. Balances have been known to maintain their characteristics for a few years.

USES OF BALANCE

The balance may be used as a laboratory microbalance. Because of the nature of its design, the balance has many applications to specific analytical and research problems.

Ash Determinations. Determinations of the metal content of organometallic compounds, or organic complexes with metals, may be carried out by direct ignition of the weighed sample on a platinum balance pan. The residue after such an ignition is then weighed as the metal (silver, platinum, gold), as the oxide (ferric, cupric, aluminum, silica, uranium, etc.), or as the sulfate (sodium, magnesium, barium, manganese, etc.).

Determination of Water in Hydrates. The balance is well adapted for determination of moisture content, water of hydration and similar studies of an equilibrium nature. These are run by following the weight changes of a sample on the balance pan in a closed system. The micro samples required by the fiber

balance are desirable, because they reach equilibrium much more quickly than do larger samples. The use of the balance in such a determination is best shown by an example.

Figure 8 is the well-known graph of the vapor pressure plotted against water of hydration of the various hydrates of magnesium sulfate. To illustrate a use of the balance this hydrate system was studied in the following way.

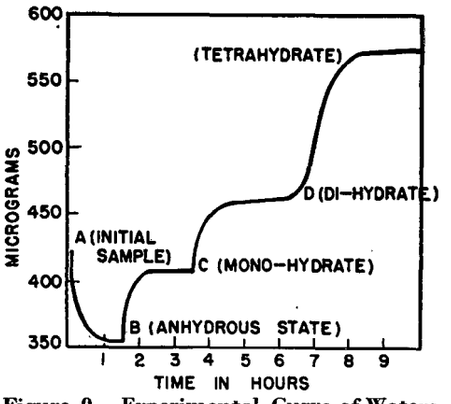

Figure 9. Experimental Curve of Waters of Hydration of Magnesium Sulfate

A sample of partially hydrated magnesium sulfate was placed on one pan of the microbalance. The balance was inserted into a closed system, the temperature of which was fixed near room temperature. A trap containing water was in the system. By using appropriate baths, the temperature of this trap was fixed at values lower than the temperature of the rest of the system. The vapor pressure of water in the closed system was thus established by the temperature of the coldest part of the system, which was the trap. Changing the temperature of the trap changed the vapor pressure of water in the system.

Changes in the weight of the sample could be interpreted in terms of hydrate formation, as shown in Figure 9. At time *A* on the curve a dry ice–acetone bath was placed around the trap, and the system was evacuated and closed. A rapid loss of weight resulted until the anhydrous state was reached. At time *B* an ice bath at a temperature of 0° C. was placed around the trap, producing a water vapor pressure of 4.6 mm. inside the system. The weight of the sample increased by an amount corresponding to the monohydrate. In like manner at times *C* and *D* baths of partially frozen dioxane and acetophenone, having temperatures of 8.6° and 16.6° C., respectively, were placed around the trap. Breaks corresponding to the formation of di- and tetrahydrates were obtained. With a salt of undetermined hydrate composition, a larger number of trap temperatures would be necessary for a complete hydrate study. To extend the temperature range, the system, excluding the trap, can be fitted with jackets through which a heated bath liquid may be circulated.

Determination of Gas Density. The balance may be used in the determination of gas densities by mounting a buoyancy bulb on one end of the beam.

Reactions in Closed Systems. Reactions may be carried out on the pans of the microbalance in closed systems under wide conditions of temperature and pressure and monitored by following the accompanying weight changes. Any gaseous reagent tolerated by the all-quartz construction may be used.

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Low Temperature Separation of Ethane from Methane and Air

ROBERT L. SLOBOD, *The Atlantic Refining Co., Dallas, Tex.*

IN THE literature (*2*, *3*, *5*, *7*) the separation of ethane from air or other more volatile substances is generally assumed to be accomplished in a satisfactory manner by the use of liquid air or liquid nitrogen. Laboratory tests using high vacuum apparatus similar to that described by Prescott and Morrison (*6*) show, however, that the use of liquid air as a refrigerant results in a considerable loss of ethane and that even at the colder temperature produced by liquid nitrogen ethane is not quantitatively retained in the cold trap. The vapor pressure of ethane, however, is sufficiently lowered when the temperature is reduced from the liquid nitrogen boiling point (−196° C.) to −210° C. to enable quantitative separation to be made. Temperatures below −196° C. can be produced by maintaining liquid nitrogen at reduced pressure, the lower limit (−210° C.) corresponding to the triple point where the system has a vapor pressure of 96.4 mm. (*4*). No attempt was made to obtain temperatures below −210° C. because the formation of solid nitrogen complicates the procedure below this point. It is obvious, therefore, that maintaining liquid nitrogen at a reduced pressure to produce a temperature approaching −210° C. offers a possible means for the quantitative condensation of ethane.

EXPERIMENTAL

The inability of liquid nitrogen at atmospheric pressure (−195.8° C.) to lower the vapor pressure of ethane to an insignificant value is demonstrated by the following experiment.

A known amount of pure dry ethane, approximately 25 cu. mm., was confined in a glass trap, which was part of an elaborate high vacuum system in which mercury cutoffs were used in place

In geochemical methods of prospecting for oil, gas samples containing methane, ethane and heavier hydrocarbons, and air are frequently obtained in which the ethane and heavier hydrocarbon fraction must be determined. A method has been developed using liquid nitrogen at reduced pressure to separate the ethane quantitatively from the methane and air. In order to carry out this procedure conveniently, a special trap has been designed incorporating a low temperature reservoir containing liquid nitrogen maintained at reduced pressure. This low temperature source cools a container of liquid nitrogen open to the atmosphere for easy accessibility. A broader application in the field of low pressure gas analysis is suggested by the data obtained showing an incomplete recovery of pure ethane from the gas phase when liquid nitrogen at atmospheric pressure is used as the refrigerant. Consequently, liquid nitrogen at reduced pressure is suggested for any separation of ethane where complete recovery is required.

of stopcocks to avoid contamination or loss in the handling of small hydrocarbon samples. While the ethane sample was maintained at approximately −196° C. by a liquid nitrogen bath, the sample was exposed directly to the pumps. Under these conditions in which the pressure over the ethane was reduced to a fraction of a micron by the mercury diffusion pump, ethane was lost at an appreciable rate, amounting to approximately 14% of the sample in the first 10 minutes of pumping. Continued pumping over a 25-minute interval showed this loss to be approximately linear with time, as shown in Figure 1.

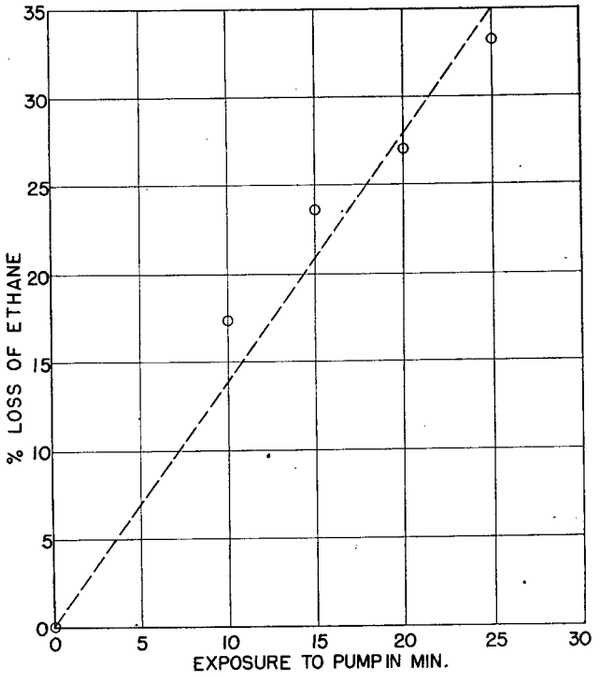

Figure 1. Loss of Ethane at Liquid Nitrogen Temperature (−196° C.) during Pumping

The ability to retain ethane quantitatively in a cold trap as a result of a lowered vapor pressure through the use of a lower temperature is demonstrated by the results of the following experiment.

A small volume (3.7 cu. mm.) of pure dry ethane was transferred to the cold trap and cooled to approximately −208° C. by using liquid nitrogen at reduced pressure as the refrigerant. This sample was then exposed to the pumps for three 5-minute intervals under pumping conditions similar to the previous test.

The quantitative retention of the ethane is shown by Table I, which also includes additional data confirming this result.

APPARATUS

The apparatus used to obtain the desired temperature of approximately −208° C. is shown in Figure 2.

The central tube, *A*, is filled with liquid nitrogen, *B*, which acts as a bath material and has the advantage of being at atmospheric pressure. Additional liquid nitrogen, *C*, is placed in the annular space between *A* and the Dewar flask, *D*. The annular space is sealed by a cork, *E*, providing a closed chamber which is evacuated through line *F*. Upon evacuation through *F*, the temperature of liquid nitrogen, *C*, is reduced to approximately −208° C. when a vacuum of about 25 to 27 inches of mercury is maintained. This large bath of cold liquid nitrogen cools the liquid nitrogen, *B*, in tube *A* to approximately −208° C. An important feature in this design, leading to great convenience in the experimental work, is the fact that the cooling bath, *B*, is maintained at atmospheric pressure, but at a temperature lower than can ordinarily be obtained with liquid nitrogen at this pressure.

Table I. Quantitative Retention of Ethane at −208° C.

Vol. of Pure Ethane at Start	Time of Pumping with Diffusion Pump	Vol. of Ethane after Pumping	Result
Cu. mm.	*Min.*	*Cu. mm.*	
3.7	5	3.7	No loss
3.7	5	3.7	No loss
3.7	5	3.7	No loss
5.8	2	5.8	No loss
5.8	2	5.8	No loss
5.8	2	5.8	No loss

DISCUSSION

The problem of quantitatively separating ethane by condensation from more volatile constituents is complicated by the fact that ethane has a small but significant vapor pressure at the temperature of liquid nitrogen (−195.8° C.). The equation (*1*) for the vapor pressure of ethane as a function of temperature is

$$\log P = -1050.8/T + 1.75 \log T - 0.0134T + 7.102$$

where P is in millimeters of mercury, T is absolute temperature in degrees Kelvin, and the log is to the base 10. Assuming this equation to apply over the temperature range of interest, the vapor pressure at −196° C. (77° K.) is calculated to be 5.4 ×

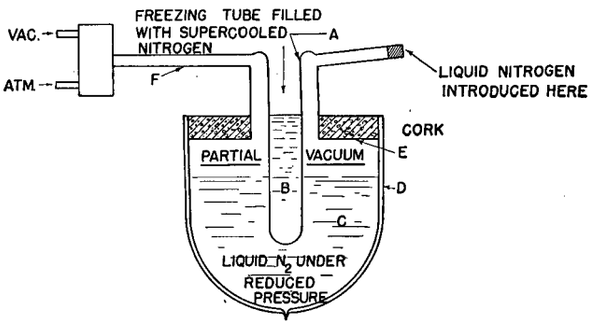

Figure 2. Apparatus for Producing Low Temperature
Using liquid nitrogen at reduced pressure

$10^{-2}\mu$. This value, although low, is significant in a vacuum system where the total pressure may easily be only one fifth of this number. Lowering the temperature of the freezing trap, therefore, is the obvious solution to prevent loss of ethane. From the above equation, the calculated vapor pressure of ethane at −210° C. (63° K.) is $5.3 \times 10^{-5}\mu$, which is only 0.001 of the vapor pressure at −196° C. Experimental verification of this significant lowering of the vapor pressure is given in Table I, where the data show that no loss of ethane was observed when the condensed hydrocarbon was exposed to the pumps at this low temperature obtained by maintaining liquid nitrogen at a reduced pressure of 25 to 27 inches of mercury.

This method (cooling at −210° C.) has been used to recover ethane quantitatively from air mixtures containing small amounts of methane.

The gas mixture was passed through a coiled glass trap placed in the refrigerated zone, *B* (Figure 2). To effect complete removal of the condensable fraction, the gases were circulated with a Toepler pump for at least five passes over the trap. The noncondensed gases (methane and air) were removed by exposing the system to the pumps. The amount of condensed gas was determined by warming the trap and pumping the gas into a measuring pipet identical with that described by Prescott and Morrison (*6*). The measured sample was then transferred to a sample tube by circulating the gas over a 3-mm. glass tube immersed in liquid nitrogen at approximately −210° C. The gas was sealed off in the sample tube and then introduced into a mass spectrometer where the amounts of ethane, methane, and air were determined.

In all cases the sample was found to be essentially free of methane. In some instances a few per cent of methane was reported, but the amount was comparable with the experimental error of the determination. It is conceivable that in the presence of large amounts of methane more of this gas might be present in the condensed fraction with the ethane. Such contamination could be reduced by vaporizing the sample and then recondensing the ethane at −210° C., leaving the uncondensed methane to be pumped off.

Because no other refrigerant is known which can be used to produce conveniently a temperature of approximately −210° C., liquid nitrogen at reduced pressure seems to offer a unique solution to the problem of obtaining simply the conditions needed for the quantitative recovery of ethane from air and methane.

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The author wishes to acknowledge the assistance in the analytical work provided by Mrs. M. E. C. Keiles.

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Kjeldahl Microdigestions in Sealed Tubes at 470° C.

LAWRENCE M. WHITE AND MARION C. LONG
Western Regional Research Laboratory, Albany, Calif.

Kjeldahl digestions for the microdetermination of heterocyclic nitrogen were carried out in heavy-walled, sealed glass tubes at 470° C. with concentrated sulfuric acid and mercuric oxide catalyst. At this temperature the digestion is complete in a fraction of the time usually recommended for heterocyclic compounds. The accuracy and precision of the method are good because there is no possibility of nitrogen loss due to thermal decomposition of ammonium bisulfate or by bumping. The pressure developed within the digestion tubes is nominal.

IN 1889 Gunning (*5*) introduced the use of potassium sulfate to hasten the Kjeldahl digestion by raising the boiling temperature of the digest. Its use has become general. The increased boiling temperature is especially effective in shortening the time required to obtain complete nitrogen recovery from refractory compounds. Thus Ogg and Willits (*7*) showed that the time required for complete digestion of nicotinic acid was approximately halved for each 10° C. the temperature of the digest was raised, and even with as much as 625 mg. of potassium sulfate per milliliter of sulfuric acid, they found that a minimum of 3 hours was required for the complete digestion of this refractory material on the microscale.

Too high a concentration of potassium sulfate has been shown to cause nitrogen loss due to thermal decomposition of the ammonium bisulfate formed during the digestion. Therefore, there is a practical limit to which the digestion time may be shortened by increasing the boiling temperature of the digest through the addition of potassium sulfate.

To avoid the uncertainties incident to use of high concentrations of potassium sulfate and the long, tedious digestion required for refractory materials, the authors digest such samples with sulfuric acid and mercuric oxide in sealed tubes at 470° C. Under these conditions nitrogen cannot be lost through thermal decomposition of ammonium bisulfate or by bumping. The digestion requires little attention and it is completed very quickly. A previously described Kjeldahl sealed-tube digestion macromethod (*6*), using fuming sulfuric acid and requiring several hours' heating at 330° C., does not offer the advantages of speed and convenience inherent in the method described here.

PROCEDURE

Weigh a 5- to 10-mg. sample into a heavy-walled borosilicate glass Carius tube (*9*) approximately 7 inches (17.5 cm.) long. Add 40 mg. of mercuric oxide and 1.5 ml. of concentrated sulfuric acid and seal the tube with a gas-oxygen torch. Place the sealed tube in an inclined position on a corrugated aluminum shelf in a welded steel box constructed to fit closely within a temperature-controlled muffle furnace. Close the box and insert it into the muffle heated to 560° C. and reset the temperature control to 470° C., the desired digestion temperature. (The heat capacity of the box made it necessary to preheat the muffle to 560° C., so that the sample would quickly come to temperature. Under these conditions, the shelf that supported the tubes reached 470° C. in approximately 15 minutes.) Heat 15 minutes at 470° C.,

Table I. Analyses of Nitrogen-Containing Compounds

Compound	Type of Nitrogen	Nitrogen Content, % Theory	Average	Replicates
Cystine, N.B.S. No. 143	Amine	11.66	11.61	11.63, 11.63, 11.60, 11.60, 11.59
Acetanilide, N.B.S. No. 141	Amide	10.36	10.32	10.34, 10.32, 10.32, 10.31, 10.30, 10.30
l-Lysine hydrochloride	Amine	15.34[a]	15.41	15.44, 15.40, 15.39
Nicotinic acid	Pyridine	11.38	11.42	11.46, 11.43, 11.42, 11.40, 11.37
2,2′-Bipyridine	Pyridine	17.94	17.87	17.92, 17.85, 17.84
4-Pyridylpyridinium dichloride	Pyridinium and pyridine	12.23	12.11	12.21, 12.10, 12.09, 12.05
dl-Tryptophan	Indole and amine	13.72[b]	13.67	13.68, 13.66
8-Hydroxyquinoline	Quinoline	9.65	9.56	9.58, 9.57, 9.52
Histamine dihydrochloride	Diazole and amine	22.83	22.86	22.91, 22.86, 22.82
Sulfathiazole	Thiazole, amine and amide	16.46	16.35	16.39, 16.33, 16.32
Uric acid	Purine	33.33[c]	33.13	33.20, 33.16, 33.11, 33.03
Quinine sulfate	Quinoline and 1-azabicyclooctane	7.50	7.45	7.49, 7.44, 7.42
Quinidine sulfate	Quinoline and 1-azabicyclooctane	7.50	7.51	7.53, 7.51, 7.50
Strychnine sulfate	Indole or dipyrrole	7.31	7.25	7.26, 7.25, 7.23
Brucine sulfate	Indole or dipyrrole	6.31	6.23	6.26, 6.22, 6.21
Atropine sulfate	Nortropane	4.14	4.14	4.14, 4.14, 4.14
4-Nitroacetanilide	Nitro and amide	18.66	11.15	11.16, 11.15, 11.14
Aminopyrine	Pyrazolone and amine	18.17	14.72	14.83, 14.76, 14.58

Nitrogen content by routine Kjeldahl micromethod with:
[a] 110-min. digestion, 15.40% (*11*).
[b] 360-min. digestion, 13.70% (*11*).
[c] 80-min. digestion, 33.14%.

remove the box from the muffle, and allow it to cool slowly or place it under a multiple-jet air blast to cool it quickly. After the box reaches room temperature, remove the tube and open it at room temperature as previously described (*9*). Dilute the contents of the tube with 2 to 3 ml. of water and transfer it to a 50-ml. beaker with 8 to 10 ml. of water. When cool, wash the sample into the Kjeldahl still containing 8 ml. of a solution of 40% w./v. sodium hydroxide and 5% w./v. sodium thiosulfate. Distill and titrate the ammonia by any standard procedure.

RESULTS

The analyses of National Bureau of Standards cystine and acetanilide, lysine, a group of heterocyclic nitrogen compounds, and representative samples requiring reduction before the Kjeldahl digestion are presented in Table I. Many of the compounds listed have not been reported to be refractory, but they have been included in this study to show that the method is effective for a variety of nitrogen-containing rings.

Tryptophan and lysine are among the compounds usually regarded as being refractory. The results for these amino acids are in good agreement with theory and with the values previously found for these samples (*11*) by routine Kjeldahl microdeterminations with extended digestion.

The refractory nature of nicotinic acid is probably due to the presence of nitrogen in the heterocyclic pyridine ring. Pyridine-type nitrogen was determined satisfactorily by the present method in nicotinic acid, 2,2′-bipyridine, and 4-pyridylpyridinium dichloride. Compounds containing a variety of other heterocyclic nitrogen structures were analyzed successfully (see Table I). Amine and amide nitrogen were also recovered quantitatively.

Clark (*2*) reported that atropine and quinine required extended digestion, and Drevon and Roussin (*3*) found that quinine was not properly analyzed under their conditions. Nevertheless, these and the other alkaloids listed in Table I were analyzed without difficulty by the method described herein.

The data for 4-nitroacetanilide and aminopyrine show that this method cannot be used in place of the Friedrich hydriodic acid procedure (*2, 4, 8*) for nitrogen linkages requiring reduction prior to the usual Kjeldahl digestion.

The data in Table II show that the amounts of acid and catalyst used are not critical. The amounts specified in the procedure were chosen so that the same reagent dispensers could be used for this and for the Clark (*2, 10*) method routinely used in this laboratory. The data in this table also show that the digestion is complete after 7 minutes of heating at 470° C., when mercuric oxide is used as catalyst, but that 30 minutes are required in the absence of catalyst. A 15-minute digestion with catalyst was chosen to provide an ample margin of safety.

DISCUSSION

The relatively high temperature employed, about 120° C. higher than that practicable in the open flask, permits the rapid and complete digestion of refractory compounds without loss of any free ammonia which might be generated by dissociation of ammonium bisulfate. The method should also be useful for the analysis of volatile samples or those that form volatile nitrogenous products during digestion. The complete digestion of the sample after 30 minutes' heating at 470° C. without catalyst (Table II) suggests that this method should be useful when other constituents (sodium, potassium, phosphorus, etc.) must be determined in addition to nitrogen.

The sealed-tube method of digestion requires no new techniques, because the sealing and opening of the tubes are the same as for the Carius microprocedures and the distillation is the same as that usually used, except that the base must be added to the still before the sample. This is required because the carbon dioxide, sulfur dioxide, and other acid gases formed during the digestion are not driven off as they are during the open-flask digestion. Addition of the base to the still prior to the sample is recommended for the conventional procedure by Belcher and Godbert (*1*).

The welded steel box was used to permit the digestion of several samples at a time and as a safety shield. The corrugated shelf that supports the tubes was made of aluminum to provide rapid heat transfer to the tubes and to promote even temperature throughout the box. In preliminary work the tubes were heated in 8-inch lengths of 0.5-inch black iron pipe provided with two screw caps and a small vent hole near the top.

Table II. Effect of Length of Digestion and Amounts of Reagents on Recovery of Nitrogen from Nicotinic Acid

Time Digested at 470° C.[a]	Sulfuric Acid	Mercuric Oxide	Nitrogen Found %	Nitrogen Found % of theoretical
Min.	*Ml.*	*Mg.*		
1	0.5	13	9.42, 8.96	82.8, 78.7
	0.5	0	2.53, 2.83	22.2, 24.9
	1.5	40	7.66, 8.52	67.3, 74.9
	1.5	0	4.40, 3.94	38.7, 34.6
	0.5	13	11.38, 11.43	100.0, 100.4
	0.5	0	9.85, 9.73	86.6, 85.5
	1.5	40	11.37, 11.41	99.9, 100.3
	1.5	0	8.63, 8.81	75.8, 77.4
15	0.5	13	11.38, 11.43	100.0, 100.4
	0.5	0	9.85, 10.63	86.6, 93.4
	1.5	40	11.39, 11.41	100.1, 100.3
	1.5	0	10.18, 9.77	89.5, 85.9
30	0.5	13	11.39, 11.39	100.1, 100.1
	0.5	0	11.29, 11.48	99.2, 100.9
	1.5	40	11.44, 11.41	100.5, 100.3
	1.5	0	11.35, 11.33	99.7, 99.6

[a] Temperature of shelf that supported tubes.

The pressure resulting from the digestion of the 5- to 10-mg. samples specified should amount to only a few atmospheres. That the heavy-walled tubes used are amply strong to withstand the pressure is demonstrated by the fact that not a single tube failed at any time during this investigation. In order to test the tubes further, 50-mg. samples of dextrose and 33-mg. samples of corn oil were digested at temperatures up to 525° C. for several hours. This is 55° C. above the temperature specified for the method and 15° C. above the strain point of the glass used. Samples of this size at the higher temperature generate very much more pressure than do the 5- to 10-mg. samples at the temperature used in the method. Even under these extreme conditions, there was still no failure of the tubes.

As a safety precaution, commercial Carius microtubes should not be used for this procedure and the tubes should come to room temperature before they are removed from the shield and opened. If desired, the digestion box can be opened behind a safety glass shield, and the pressure within the tubes can be released before they are removed from the box by applying a small, sharp flame to the tips of the tubes until they open as a result of the slight internal pressure.

The results obtained with this method show excellent precision and accuracy, and both working and elapsed times are very favorable when compared with the long digestion procedures often recommended for heterocyclic or refractory nitrogen compounds.

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Microscopic Fusion Analysis of Sterols

VICTOR GILPIN[1], *Yale University, New Haven, Conn.*

A recently developed method of microscopic identification has been investigated by which minute quantities of sterols can be easily and rapidly recognized. Although there are limitations to the method, it should prove a valuable aid in the field of sterol analysis.

IN RECENT years, considerable interest has been shown in the identification of compounds by means of microscopic fusion methods. Although all such methods depend on the same phenomena, two differing techniques have been developed. For convenience, these may be called the Kofler method, in which a hot stage is used (*3*), and the McCrone method, in which a hot stage is not used (*1, 4*). The latter method has the advantages of greater speed and simplicity. Almost all the data on compounds studied by the McCrone technique appear as a part of a large crystallographic program (*5*). It is the aim of the present study to explore the possibilities of the McCrone analysis as an independent means of identification of related compounds. The sterols are excellent materials for this work, because they melt at moderate temperatures, usually without decomposition.

To describe interference figures where no optic axis is visible the following terms have been employed: indefinite—for relatively clear figures, from single crystals, whose orientation cannot be definitely specified; one brush—for relatively clear figures in which one isogyre sweeps the field on rotation of the stage; diffuse—for a conoscopic view consisting of superimposed figures, the result of several crystals in the field at once.

Not all observations yield useful data for all compounds—for example, heating some compounds gives rise to no sublimation, decomposition, or mesomorphs ("liquid crystals"); in such cases, observations on heating the solid are omitted. Again, if the crystallization velocity of the solidifying melt change with temperature is not reported, it is to be inferred that such change is slight. Omission of the optic sign and estimate of $2V$ from interference figure descriptions implies that the figures are not sharp enough for reliable determination of these properties. All conoscopic observations were made with a 0.85 numerical aperture dry objective; all photographs and observations (except refractive index) were made with crossed Nicols.

CHOLESTEROL

Cooling and Solidification of Melt. The melt supercools slightly. A microcrystalline mass solidifies spontaneously and moves rapidly into melt. During the cooling period, the crystallization velocity abruptly decreases and parallel or curving blades and rods appear; meanwhile, the crystal front changes from smooth to serrated. On further cooling, the crystallization velocity increases slightly. Transverse shrinkage cracks appear soon after complete solidification.

Partially Remelting Solidified Melt ("Melt-back"). Bladelike crystals grow (Figure 1).

Solidified Preparation. The microcrystalline solid often shows a herringbone pattern (Figure 1). Bladelike crystals show nearly parallel extinction, negative elongation, low birefringence, and nearly centered $O.A.$ or off-center Bx_a (optic sign positive, $2V$ large).

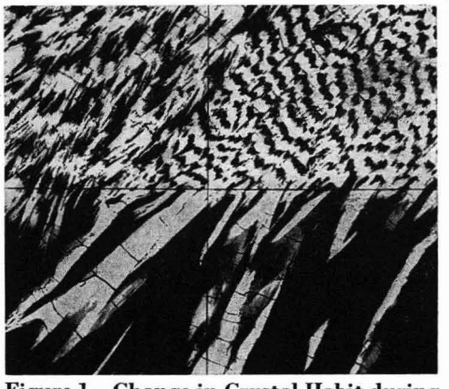

Figure 1. Change in Crystal Habit during Solidification of Cholesterol

Mixed Fusion of Compound with Thymol. Blades and needles at boundary show poor profile angles; one refractive index equals melt, and one refractive index is greater than melt. After a few minutes, nuclei of a new phase, possibly an addition compound, appear at the boundary. The bladelike crystals of this

[1] Present address, Department of Chemistry, Michigan State College, East Lansing, Mich.

phase show very low birefringence, positive elongation, and both refractive indices slightly greater than melt.

CHOLESTERYL ACETATE

Cooling and Solidification of Melt. There is no supercooling. A fine-grained, uniform, gray mesomorph grows at moderate crystallization velocity. Pressure applied to the cover glass causes the mesomorph to turn a vivid rust color. This, and other mesomorph colors, can usually be seen equally well with the naked eye by reflected light. From several nuclei of the solid, circular spherites with a smooth circumference grow at medium crystallization velocity. As the temperature falls, the crystallization velocity slows to nearly zero, and, at the same time, the number of solid nuclei increases tremendously (Figure 2).

Solidified Preparation. The radiating hairlike crystals in the spherites show fairly low birefringence, parallel extinction, negative elongation, and a diffuse interference figure.

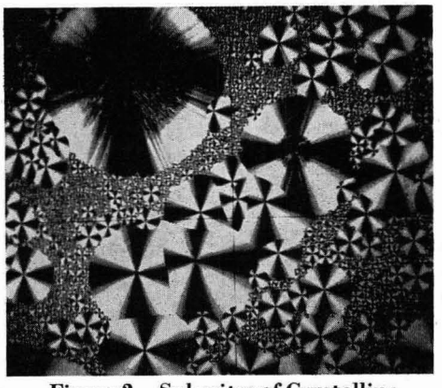

Figure 2. Spherites of Crystalline Cholesteryl Acetate

CHOLESTERYL PROPIONATE

Heating the Solid. The solid melts to a bright blue mesomorph showing white streaks. Further heating gives the isotropic liquid.

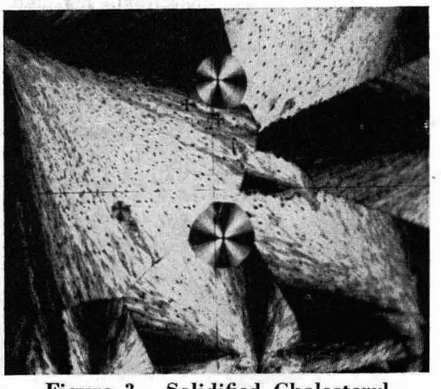

Figure 3. Solidified Cholesteryl Propionate

Round spherites–unstable polymorph

Cooling and Solidification of Melt. There is no supercooling. A fine-grained, gray-white mesomorph moves with high crystallization velocity. Pressure applied to the cover glass causes the mesomorph to turn bright blue, which changes first to bright green and later to rust color as the preparation cools. Two types of solid soon appear from numerous nuclei: (*a*) fine needles, in spherites of an unstable polymorph, showing very low birefringence and relatively low crystallization velocity (Figure 3); (*b*) larger areas of narrow blades of the stable polymorph, showing relatively higher crystallization velocity and birefringence than (*a*) (Figure 3). Strings of small air bubbles are often evident in (*b*). The transformation velocity of solid polymorphs is nil at room temperature, but the transformation known as boundary migration (*4*) gradually changes the appearance of the stable polymorph.

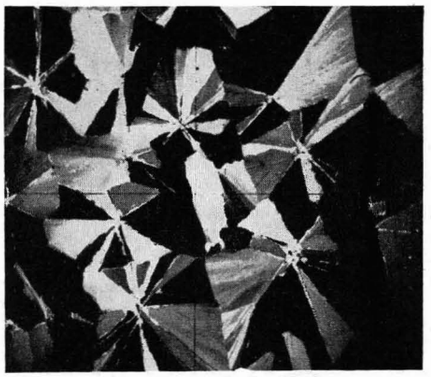

Figure 4. Solidified Cholesteryl Benzoate

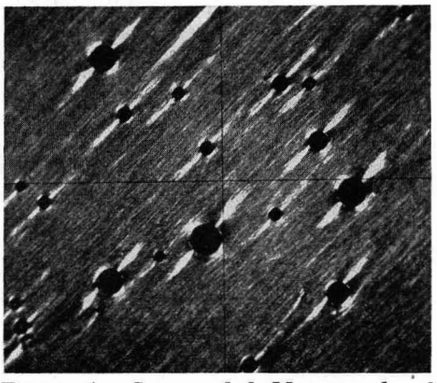

Figure 5. Supercooled Mesomorph of Cholesteryl *o*-Iodobenzoate Showing Air Bubbles

Solidified Preparation. Radial needles in the unstable spherites show parallel extinction, negative elongation, and a diffuse interference figure. Crystals of the stable polymorph show nearly parallel extinction, negative elongation, and a diffuse interference figure.

Mixed Fusion with Thymol. From the stable polymorph, stubby, slightly curved teeth grow into the melt. One refractive index equals melt; the other is slightly greater than melt.

CHOLESTERYL BENZOATE

Heating the Solid. It melts to a bluish mesomorph with scattered white streaks. Further heating gives the isotropic liquid.

Cooling and Solidification of Melt. There is no supercooling. A fine-grained, white mesomorph moves at medium crystallization velocity across the preparation. Pressure on the cover slip causes the mesomorph to become a gray color that gradually changes to bright blue. The solid appears as fairly large bright areas. The serrated crystal front moves at a medium crystallization velocity which increases on cooling. Just above room temperature, the solidification is rapidly completed by the appearance of many rectangular-shaped nuclei, which grow rapidly. Some crystals develop fine shrinkage cracks.

Solidified Preparation. Elongated crystals show close to 25° extinction, and medium to high birefringence (Figure 4). Almost all crystals show a centered indefinite interference figure.

Mixed Fusion with Thymol. Spikes and dendrites grow into the melt. These show approximately parallel extinction, negative elongation, poor profile angles, and low contrast with the liquid.

CHOLESTERYL *o*-IODOBENZOATE

Heating the Solid. It melts to a grayish mesomorph showing white streamers. Further heating gives the isotropic liquid.

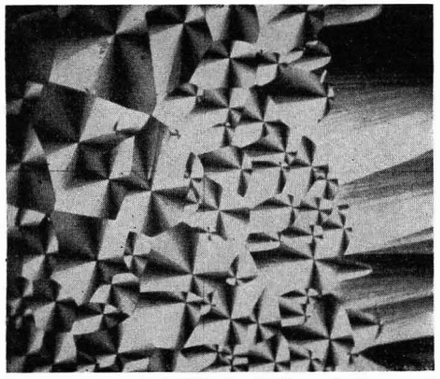

Figure 6. Solidified 7-Ketocholesteryl Acetate

Cooling and Solidification of Melt. A fine-grained, white mesomorph moves slowly across the preparation. The mesomorph supercools to room temperature, and does not solidify when seeded with solid. When the cover slip is moved the appearance of the preparation changes to gray with white streamers. The white streamers, especially noticeable near air bubbles (Figure 5), show parallel extinction, positive elongation, and an indefinite, centered interference figure.

7-KETOCHOLESTERYL ACETATE

Cooling and Solidification of Melt. The melt supercools slightly. A smooth continuous crystal front grows with medium crystallization velocity which increases on cooling. Spherites often form (Figure 6).

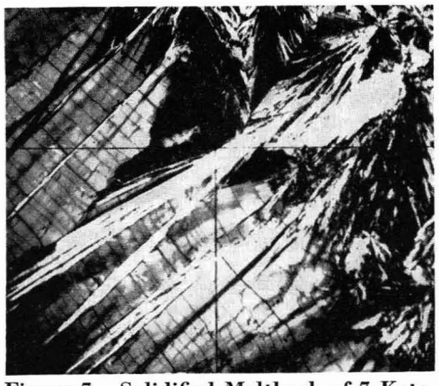

Figure 7. Solidified Meltback of 7-Ketocholesteryl Benzoate, Showing Transverse Shrinkage Cracks

Solidified Preparation. Fine hairlike crystals show extremely low birefringence; the polarization colors are nearly always dark gray. The extinction is oblique at about 45°.

Mixed Fusion with Thymol. A smooth gray front with a whitish border forms at the interface. The contrast between solid and melt is very low.

7-KETOCHOLESTERYL BENZOATE

Heating and Solid. The solid melts to a bluish mesomorph showing white streamers. Further heating gives the isotropic liquid.

Cooling and Solidification of Melt. The melt supercools slightly. A fine-grained, white mesomorph slowly covers the preparation. The crystalline solid, with a fairly smooth front, moves with a medium crystallization velocity which increases slightly on cooling. Just above room temperature, several coarse spherules complete the solidification.

Meltback. Broad blades of very low birefringence are formed, interspersed with a few highly birefringent needles (Figure 7). Prominent transverse shrinkage cracks appear soon after solidification.

Solidified Preparation. Highly birefringent needles show parallel extinction and negative elongation. The interference figure varies from indefinite, centered, to one brush. Low birefringent areas all show centered Bx_a (optic sign negative, $2E = 39°$, no dispersion).

Mixed Fusion with Thymol. Sharply pointed, highly birefringent needles and stubbier, low birefringent blades grow at the melt boundary. The needles show 40° to 50° profile angles, the blades about 60° to 70°. Needles have one refractive index slightly less than melt, one refractive index greater than melt, and a diffuse interference figure. Blades show both refractive indices slightly less than melt, and a centered Bx_a figure. Both habits exhibit parallel extinction and negative elongation.

7(β)-HYDROXYCHOLESTERYL BENZOATE

Cooling and Solidification of Melt. The preparation solidifies to an isotropic glass.

Figure 8. Growing Crystal Front of 7(β)-Hydroxycholesteryl Benzoate

Meltback. Seeding with the solid and careful reheating just below the melting point results in slow growth of sheaves of needles and narrow blades (Figure 8). Transverse shrinkage cracks appear on cooling.

Solidified Preparation. Needles show parallel extinction, negative elongation, one refractive index equal to melt, and one refractive index slightly greater than melt.

i-CHOLESTERYL METHYL ETHER

Cooling and Solidification of Melt. The melt supercools to a viscous liquid. On seeding with solid, fan-shaped sheaves of needles and laths grow very slowly. The needles gradually coalesce to a fine-grained, smooth front.

Solidified Preparation. The original needles show parallel extinction, negative elongation, and both refractive indices

greater than melt. In later stages of solidification the solid exhibits a peculiar characteristic herringbone appearance (Figure 9).

DICHOLESTERYL ETHER

Heating the Solid. The solid melts to a milky mesomorph, then almost immediately to the isotropic liquid.

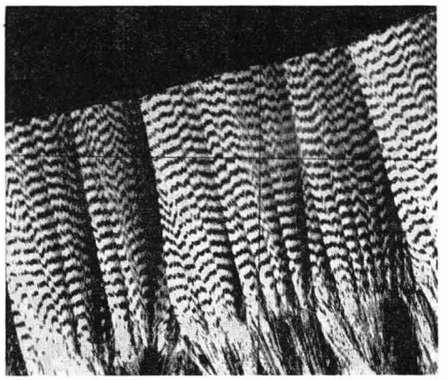

Figure 9. Growing Crystal Front of *i*-Cholesteryl Methyl Ether

Cooling and Solidification of Melt. There is no supercooling. A relatively coarse-grained and highly birefringent mesomorph forms rapidly. Solid forms quickly, and the fairly smooth front moves with medium crystallization velocity which increases rapidly on cooling. A microcrystalline mass often completes the solidification. Complex shrinkage cracks appear.

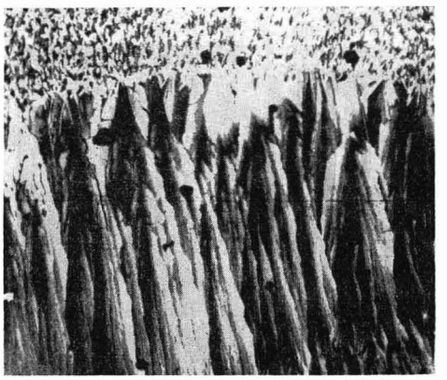

Figure 10. Solidified Dicholesteryl Ether

Solidified Preparation. Fairly large areas of roughly uniform color show a ropy or rippled appearance when rotated to extinction (Figure 10). The interference figure is usually one brush, or an off-center bisectrix ($2V$ large). A few small scattered areas show uniform parallel extinction and negative elongation.

Many sharply pointed needles grow in the boundary, all showing parallel extinction, negative elongation, and a nearly centered indefinite interference figure. One refractive index is slightly greater than melt and the other equals melt.

CHOLESTANE

Cooling and Solidification of Melt. The melt supercools considerably. Numerous nuclei of the stable solid appear, and grow with a barely perceptible crystallization velocity into oval or lens-shaped areas of narrow, bladelike crystals. In the melt, other nuclei of an unstable solid form grow, with a much slower crystallization velocity, into round spherites showing concentric

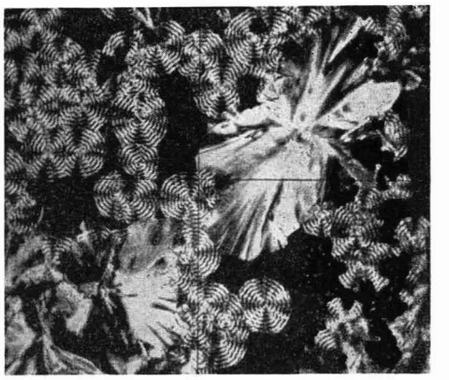

Figure 11. Round Spherites of Unstable Form of Cholestane Being Transformed on Contact with Stable Form

light and dark rings. When the two polymorphs meet, there is a very slow transformation (Figure 11).

Solidified Preparation. Smaller nuclei of the stable form show roughly uniform extinction, parallel to the long axis of the nucleus. All views show low contrast with the melt, and a nearly centered indefinite interference figure. Some large spherites have narrow-bladed crystals with 10° extinction. The unstable form shows all refractive indices slightly greater than melt; the fast ray is parallel to the spherite radius.

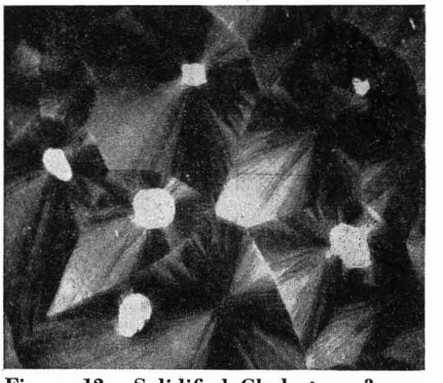

Figure 12. Solidified Cholestane-3-one Showing Low Birefringent Unstable Form and High Birefringent Stable Form

Mixed Fusion with Thymol. Thymol markedly increases the crystallization velocity of the stable form; fairly rapid solidification takes place at the boundary between the two liquids. Very thin platelike crystals of the stable form grow into the melt. These all show 90° profiles.

CHOLESTANE-3-ONE

Cooling and Solidification of Melt. The melt supercools slightly. A smooth, fine-grained solid front of extremely low birefringence moves with a medium crystallization velocity which increases slightly on cooling. Rapid chilling of the melt leads to formation of spherites of radiating hairlike crystals.

Meltback. In the melt and in the solid highly birefringent nuclei of a more stable polymorph appear (Figure 12). The crystallization velocity of the unstable form is much greater than that of the stable form. The transformation velocity slows to zero at room temperature.

Solidified Preparation. Hairlike crystals of the low birefringent unstable form have parallel extinction and negative

elongation. Much of the stable polymorph is microcrystalline. A few larger crystals show 5° extinction, positive elongation, and a diffuse interference figure.

Mixed Fusion with Thymol. The smooth front at the boundary of the thymol and the unstable form is invisible except between crossed Nicols. A few tiny rods grow from the stable form. These show 19° to 25° extinction, one refractive index equals melt, and one refractive index is greater than melt. The rods rapidly dissolve in the thymol.

3(β),5(α),6(β)-CHOLESTANETRIOL

Cooling and Solidification of Melt. The melt supercools slightly. A jagged solid front of lathlike crystals moves with fairly high crystallization velocity which increases on cooling. Shrinkage cracks, usually transverse, appear in the solid.

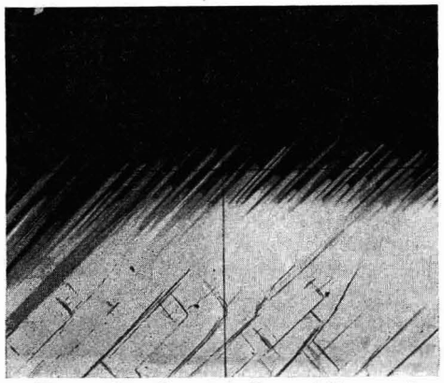

Figure 13. Growing Crystal Front of 3(β),5(α),6(β)-Cholestanetriol

Solidified Preparation. Most of the solid, consisting of broad crystals of low birefringence, shows parallel extinction, positive elongation, and varying *O.A.* views. Dispersion is negligible and the *O.A.P.* is normal to the length of the crystal. A few narrow laths of higher birefringence show parallel extinction, negative elongation, and a one brush or centered indefinite interference figure.

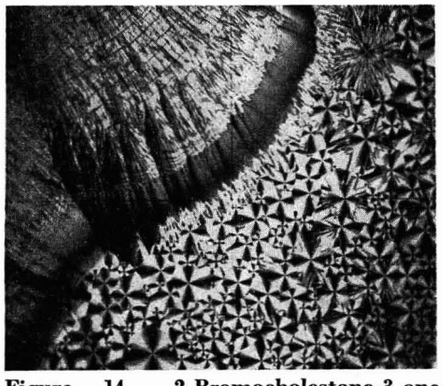

Figure 14. 2-Bromocholestane-3-one Showing Two Stages of Solidification

Mixed Fusion with Thymol. Parallel needles and laths grow into the melt (Figure 13). All show parallel extinction, and poorly defined profile angles. Some of the crystals, showing negative elongation (as above), have one refractive index very slightly less than melt and one refractive index greater than melt. The other crystals, showing lower birefringence and positive elongation (as above) have one refractive index very slightly less than melt and one refractive index considerably less than melt.

2-BROMOCHOLESTANE-3-ONE

Heating the Solid. The solid decomposes slightly if not heated carefully.

Cooling and Solidification of Melt. The melt supercools considerably. The melt solidifies fairly rapidly into a multitude of tiny nuclei.

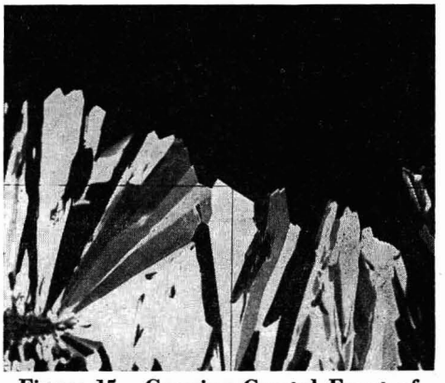

Figure 15. Growing Crystal Front of Δ^2-Cholestene

Meltback. A profusion of fine needles grows with medium crystallization velocity. The needles gradually coalesce to form a smooth solid front. Just above room temperature many small round spherites appear ahead of this front, and complete the solidification (Figure 14). Transverse shrinkage cracks appear in the original needles.

Solidified Preparation. All the needles, fine and coarse, show parallel extinction, negative elongation, and a diffuse interference figure.

Mixed Fusion with Thymol. A profusion of needles grows into the thymol, showing parallel extinction, negative elongation, a diffuse interference figure, and either 62° or 124° profiles. One refractive index is slightly less than melt, and one refractive index is greater than melt.

Δ^2-CHOLESTENE

Cooling and Solidification of Melt. The melt supercools to a viscous liquid at room temperature. On seeding, bladelike crystals gradually unite to form a sawtooth front (Figure 15) which moves with a very low crystallization velocity.

Solidified Preparation. Of several habits noticeable at the crystal front, the following are most common: (*a*) 90° profiles showing a centered Bx_a; *O.A.P.* parallel to one of the profile edges; (*b*) 73° profiles, the interference figure showing an *O.A.* at the edge of the field; optic sign negative, estimated $2V$ equals 80° (from curvature), slight dispersion r greater than v; (*c*) 40° profiles, with an interference figure showing off-center Bx_a. The extinction is close to parallel, but difficult to measure, as the sides of most crystals are not parallel.

Mixed Fusion with Thymol. The larger crystals split up at the thymol boundary into long spikes with poor profile angles; most of these show parallel extinction and a centered Bx_a, but a few show a centered *O.A.* The crystallization velocity increases noticeably in the presence of thymol.

Δ^4-CHOLESTENE-3-ONE

Cooling and Solidification of Melt. The melt supercools to a viscous liquid. After 5 to 10 minutes, numerous scattered nuclei form, and grow exceedingly slowly into round spherites (Figure 16).

Solidified Preparation. Very fine to coarse needles radiate from a common center. All needles show parallel extinction, negative elongation, one refractive index slightly less than melt, and one refractive index slightly greater than melt. Some parts of the spherites have nearly uniform orientation and a lower birefringence; these areas show a slightly off-center Bx_a (optic sign positive $2E = 84°$), with the *O.A.P.* parallel to the spherite radius. Dispersion is slight, r greater than v. More highly birefringent parts of the spherites show a diffuse interference figure.

2,4-CHOLESTADIENE

Cooling and Solidification of Melt. The melt supercools to a viscous liquid and must be seeded. A fan of blunt rods spreads out from the seed; the crystals get smaller and become interlaced to form a smooth front that moves at a very low crystallization velocity.

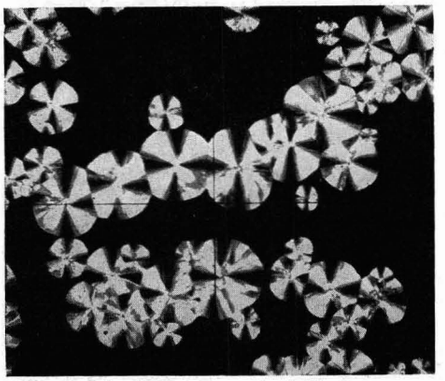

Figure 16. Spherites of Δ^4-Cholestene-3-one Growing in Melt

Solidified Preparation. Lathlike crystals show parallel extinction, and negative elongation.

Mixed Fusion with Thymol. Laths grow into the melt (Figure 17). Well-defined profile angles soon round off. The most frequently occurring habits are as follows: (*a*) square profiles, showing parallel extinction and centered indefinite interference figure; (*b*) 47° or 94° profiles showing parallel extinction, and off-center *O.A.* figure; (*c*) 54° or 108° showing bluish dispersion colors, centered *O.A.*, optic sign positive, $2V$ estimated from curvature equals 80°, moderate dispersion r greater than v. All views show refractive index greater than melt.

β-CHOLESTANYL BENZOATE

Heating the Solid. The solid melts to a metallic gray mesomorph. Further heating gives the isotropic liquid.

Cooling and Solidification of Melt. There is no supercooling. A white mesomorph appears; portions of this show relatively high polarization colors and extinction. The solid grows as clusters of long, slightly curving blades. The jagged front moves with medium crystallization velocity which increases on cooling. Just above room temperature numerous nuclei appear and complete the solidification. During cooling of the solid, the phenomenon of boundary migration is evident (Figure 18). The rate of this transformation slows to nearly zero at room temperature.

Solidified Preparation. Blades show approximately parallel extinction, negative elongation, and a diffuse interference figure.

Mixed Fusion with Thymol. Short, slightly curving, sharply pointed spikes grow at the boundary. These show varying degrees of oblique extinction, one refractive index nearly equals melt, and one refractive index is considerably greater than melt. All crystals show a diffuse interference figure.

CHOLESTERYL *p*-TOLUENESULFONATE

Heating the Solid. The solid melts to a clear liquid if heated carefully. Excessive heating produces a dark red decomposition product.

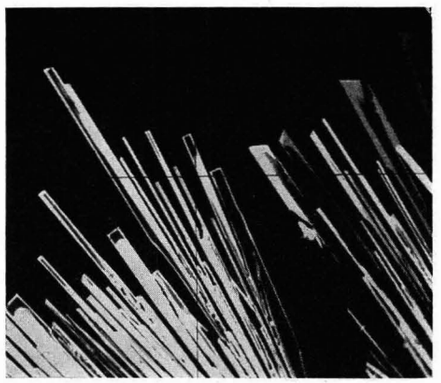

Figure 17. 2,4-Cholestadiene Growing into Thymol

Cooling and Solidification of Melt. The melt supercools to an isotropic glass.

Meltback. Rapid reheating to just below the melting point causes complete solidification as a microcrystalline white solid. Cautious heating, however, produces many fine-grained spherites of very low birefringence that show concentric light and dark circles. Many scattered nuclei of a highly birefringent polymorph also appear (Figure 19). These grow slowly at the expense of the dark form, at higher temperatures. Both the crystallization velocity and transformation velocity are zero at room temperature.

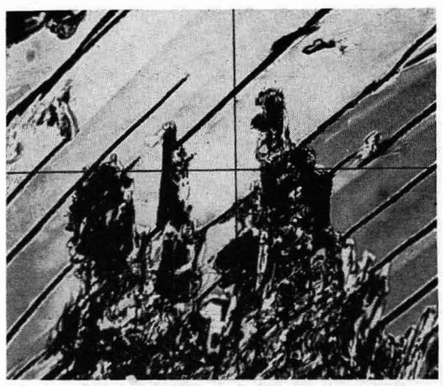

Figure 18. Solidified β-Cholestanyl Benzoate Showing Boundary Migration

Solidified Preparation. Needles of the unstable, dark form show parallel extinction, negative elongation, and a diffuse interference figure. The nuclei of the stable polymorph are usually microcrystalline; a few relatively elongated crystals show parallel extinction and negative elongation.

CHALINASTEROL

Cooling and Solidification of Melt. The melt solidifies to a glass.

Meltback. On careful reheating, many white nuclei appear. With continued heating below the melting point, many of these

enlarge into ragged spherites of fibrous, irregular needles and narrow blades (Figure 20). The crystallization velocity is zero at room temperature.

Solidified Preparation. Crystals show varying extinction, from parallel to slightly oblique, negative elongation, one refractive index equal to melt, and one refractive index slightly greater than melt.

CHALINASTERYL ACETATE

Cooling and Solidification of Melt. The melt supercools slightly. Numerous spherites of coarse, radiating needles appear during the whole solidification. Many of these coalesce into a fairly smooth crystal front which moves at a moderate crystallization velocity.

Meltback. Blades of varying width grow with an irregular front. As solidification proceeds, the blades break up into meshed needles (Figure 21), and the front becomes smooth.

Solidified Preparation. Needles in spherules have fairly low birefringence, parallel extinction, and some show positive, some negative elongation. The interference figure is diffuse. Those blades from the meltback that show uniform extinction usually have parallel extinction, positive elongation, *O.A.* at the edge of the field, optic sign positive, $2V$ large, and *O.A.P.* at right angles to the length of the blade. A few blades exhibit negative elongation, and a centered Bx_o. Many interference figures are diffuse owing to overlapping blades.

Mixed Fusion with Thymol. Thin short blades with irregular profiles and very low birefringence grow into the melt. One refractive index equals melt, and one refractive index is slightly greater than melt.

CHALINASTERYL BENZOATE

Cooling and Solidification of Melt. A fine-grained white mesomorph spreads over the melt. This gradually turns a purple color as numerous solid nuclei appear. No continuous crystal front forms. Most solid nuclei remain very small; a few grow into relatively large, leaflike crystals with irregular outlines. Distinctive curving shrinkage cracks appear (Figure 22).

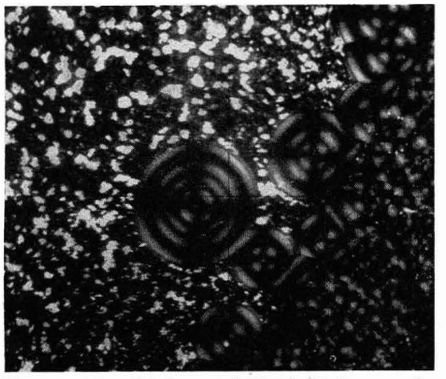

Figure 19. Light and Dark Polymorphs of Cholesteryl *p*-Toluenesulfonate

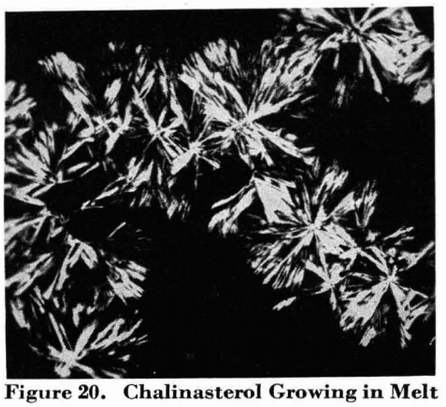

Figure 20. Chalinasterol Growing in Melt

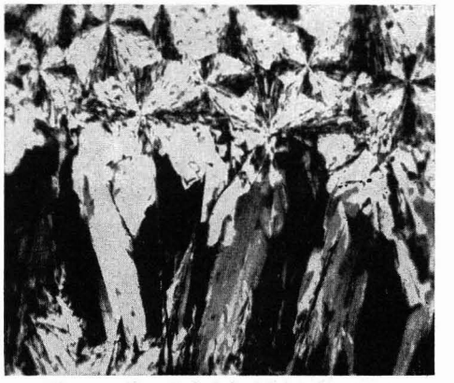

Figure 21. Solidified Meltback of Chalinasteryl Acetate

Meltback. Broad blades grow into the mesomorph. A strongly serrated front shows acute profile angles. After growing a short distance, the blades appear to curve sideways, and the rest of the melt then solidifies in nuclei as above. Cracks appear in the blades.

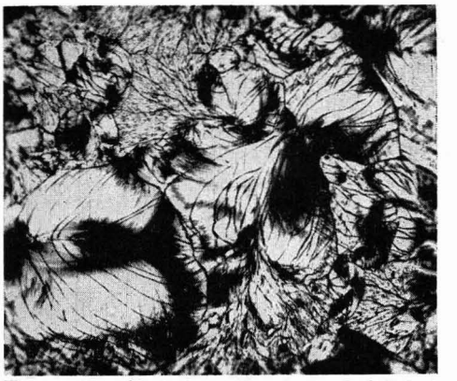

Figure 22. Shrinkage Cracks in Solidified Chalinasteryl Benzoate

Solidified Preparation. Large nuclei do not show uniform extinction. They often show *O.A.* at edge of field, but other indefinite interference figures are frequently visible. Blades from the meltback show approximately parallel extinction, varying Bx_a or *O.A.* conoscopic views, *O.A.P.* normal to long axis of the blade, optic sign positive, and $2V$ large.

Mixed Fusion with Thymol. Overlapping thin leaves, with a smooth profile, form at the boundary. There may also be groups of short, curving needles.

CHALINASTERYL PHENYLURETHANE

Cooling and Solidification of Melt. The melt solidifies to a glass.

Meltback. Before the melting point is reached, the preparation solidifies as a white, microcrystalline mass. When this solid is partially remelted, clear blade or wedge-shaped crystals grow a short distance into the melt before the crystallization velocity slows to zero as the temperature drops (Figure 23).

Solidified Preparation. Extinction is not uniform in the large crystals. Most of them, however, show an *O.A.* just outside the field, with a one brush interference figure. One refractive index is greater than glass, and one refractive index is nearly equal to glass.

PORIFERASTEROL

Cooling and Solidification of Melt. The melt supercools slightly. Many round spherules form, and coalesce to form a fairly smooth front which moves with a fairly slow crystallization velocity. A few spherules complete the solidification. Some broad areas develop complex shrinkage cracks (Figure 24).

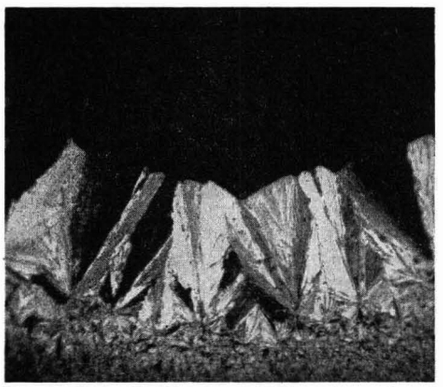

Figure 23. Chalinasteryl Phenylurethane Growing in Melt

Meltback. Below the melting point many nuclei of a more stable polymorph appear. These nuclei show very low birefringence (Figure 24), and grow rapidly. On remelting, only the stable form grows into the melt, as a profusion of roughly parallel needles, whose medium crystallization velocity increases on cooling. Transverse shrinkage cracks appear in the dark needles. The transformation velocity is zero at room temperature.

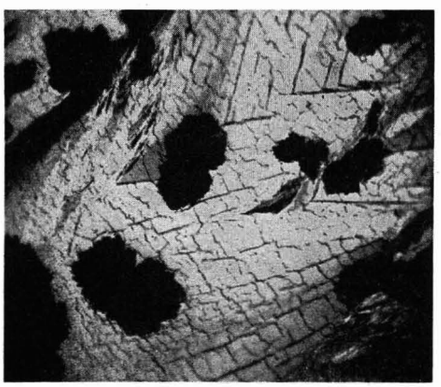

Figure 24. Dark Stable Nuclei of Poriferasterol Growing into Light Unstable Form

Solidified Preparation. The dark stable needles show parallel extinction, positive elongation, and a nearly centered, indefinite interference figure. Fine needles in the spherules of the unstable form show parallel extinction, negative elongation, and indefinite interference figure. Broader areas of the unstable form usually show a centered (indefinite) interference figure.

Mixed Fusion with Thymol. The dark, stable needles grow into the melt, but the profile angles are usually poorly defined. All needles show both refractive indices are greater than melt.

PORIFERASTERYL ACETATE

Cooling and Solidification of Melt. There is no supercooling. A slightly serrated front of blades plus a few needles grows with moderate crystallization velocity which increases slightly on cooling. A few nuclei usually appear in the melt to complete the solidification. A few transverse shrinkage cracks appear in the broader crystals.

Figure 25. Solidified Poriferasteryl Acetate

Meltback. Larger crystals form, but the behavior is no different.

Solidified Preparation. Needles show extinction varying from 0 to 20° (Figure 25). Most needles exhibit parallel extinction and negative elongation, with an *O.A.* just outside the field of view and *O.A.P.* at right angles to the long needle axis. Most blades are not single crystals, and show a diffuse interference figure. A few blades show nearly centered *O.A.*, optic sign positive, and $2V$ large.

Mixed Fusion with Thymol. Thin blades and plates of low birefringence, and thick needles of higher birefringence, show a variety of angles, of which square and 105° profiles are most common. Needles have one refractive index slightly greater than melt and one refractive index greater than melt. Plates and blades have both refractive indices slightly greater than melt.

PORIFERASTANOL

Cooling and Solidification of Melt. The melt supercools slightly. A slightly serrated front of needles and laths moves with a moderate crystallization velocity which increases on cooling. Coarse transverse or fine longitudinal shrinkage cracks appear in some parts of the solid.

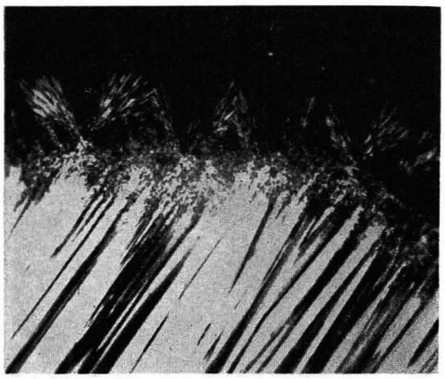

Figure 26. Poriferastanol Growing into Thymol

Solidified Preparation. Most of the solid consists of laths of very low birefringence showing slightly oblique extinction (about 5°), positive elongation, with variable *O.A.* views, optic sign positive, $2E$ large, and *O.A.P.* at right angles to the long needle axis. A few needles show higher birefringence, slightly oblique extinction, and negative elongation.

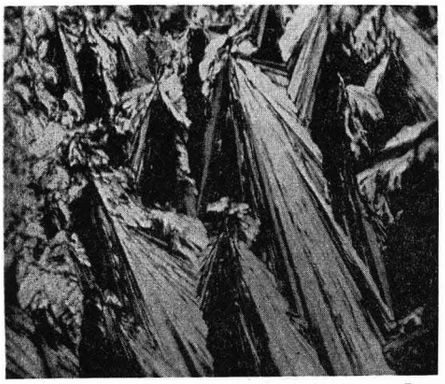

Figure 27. Solidified Poriferastanyl Acetate

Mixed Fusion with Thymol. A finely serrated front grows into the thymol. After a short time the solid-melt boundary is obscured by the solidification of many sheaves of tiny needles, which show very low birefringence and parallel extinction (Figure 26).

PORIFERASTANYL ACETATE

Cooling and Solidification of Melt. There is no supercooling. A mass of grayish nuclei forms and coalesces to give a serrated front composed of sheaves of laths and needles; the medium crystallization velocity increases slightly on cooling. Four-sided nuclei form continually in the melt during cooling. Fine longitudinal or coarse transverse cracks appear in some crystals.

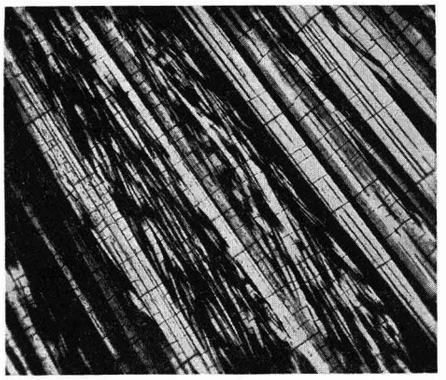

Figure 28. Laths of Stigmasterol with Shrinkage Cracks

Solidified Preparation. The first solid to form is a heterogeneous mass (Figure 27). The later crystals may be either laths of low birefringence, with parallel or slightly oblique extinction, positive elongation, and off-center Bx_a figure (optic sign positive, $2V$ large) or needles and laths of higher birefringence, showing parallel extinction and negative elongation.

Mixed Fusion with Thymol. Overlapping laths and needles, with acute profiles, grow a short distance into the melt. The more highly birefringent needles show both refractive indices greater than melt.

STIGMASTEROL

Cooling and Solidification of Melt. There is usually no supercooling. Parallel narrow laths, of low birefringence, grow with fairly fast crystallization velocity. The crystal front is not continuous, but consists of noncontiguous laths. The space between the laths freezes as an irregular matrix of randomly oriented small crystals. Prominent transverse shrinkage cracks appear (Figure 28). Occasionally there is some supercooling, and spherules appear; in addition, bright patches of an unstable, more highly birefringent polymorph are seen, but very little of this survives to room temperature where the transformation velocity is relatively slow.

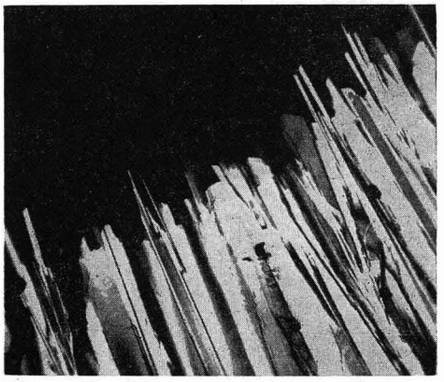

Figure 29. Stigmasteryl Acetate Growing into Thymol

Solidified Preparation. Most laths of the stable form have parallel extinction and positive elongation. The interference figure shows *O.A.* views of several orientations (optic sign positive, $2V$ large) and *O.A.P.* at right angles to the long crystal axis. A few laths show parallel extinction, negative elongation, and indefinite interference figures.

Mixed Fusion with Thymol. Laths grow into the melt showing parallel extinction, both refractive indices greater than melt, and (*a*) 73° or 146° profile angles, negative elongation, and indefinite interference figures, and (*b*) square ends, positive elongation, and indefinite interference figures. There are also many crystals with poor profiles.

STIGMASTERYL ACETATE

Cooling and Solidification of Melt. The melt supercools slightly. A few nuclei form and coalesce to give a continuous, slightly serrated front, moving with medium crystallization velocity. A few fine transverse cracks appear.

Solidified Preparation. Solid consists of blades and needles interspersed with fine-grained material of random orientation. The more frequently occurring blades show very low birefringence, parallel extinction, positive elongation, slightly off center *O.A.*, optic sign positive, $2V$ about 75° (from curvature), and *O.A.P.* at right angles to the long crystal axis. Most of the more highly birefringent needles have parallel extinction, negative elongation, and an interference figure which is usually indefinite, but occasionally may be *O.A.* at edge of field, and *O.A.P.* at right angles to the long needle axis. A few of the highly birefringent needles show 30° extinction.

Mixed Fusion with Thymol. A profusion of overlapping thin blades and needles grow into the melt (Figure 29). Most of these exhibit parallel extinction and positive elongation. Profile angles of 105° are often visible.

CAMPESTEROL

Cooling and Solidification of Melt. The melt supercools slightly. A few nuclei coalesce to form a smooth, fine-grained front, which moves with moderate crystallization velocity. On cooling further, the crystallization velocity decreases, the fine crystals grow into long, narrow laths of nearly uniform width; simultaneously the crystal front changes from smooth to slightly serrated. Transverse shrinkage cracks appear.

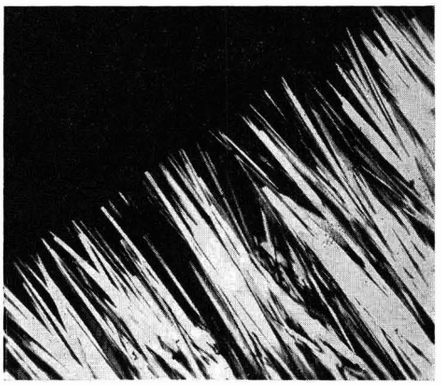

Figure 30. Campesterol Growing into Thymol

Solidified Preparation. Laths of very low birefringence show a centered *O.A.*, optic sign positive, $2V$ large, and *O.A.P* normal to the long axis of the rod. Laths of higher birefringence have parallel extinction, and either (*a*) negative elongation, with *O.A.* at edge of field, and a one brush interference figure, or (*b*) positive elongation, and varying off-center *O.A.* views.

Mixed Fusion with Thymol. Needles and narrow laths grow (Figure 30). The profile angles are acute, but are generally poorly defined in crystals broad enough to permit measurement. One refractive index is slightly greater than melt, and one refractive index is greater than melt.

CAMPESTERYL ACETATE

Cooling and Solidification of Melt. There is some supercooling. A few roughly spherulitic nuclei of radiating blades and needles unite to form a slightly serrated front which moves slowly. Occasional rough spherules form all during cooling of the melt. Complex fine shrinkage cracks usually appear in the broader crystals.

Meltback. Interlaced blades and needles grow with a slightly serrated front which gradually becomes nearly smooth.

Solidified Preparation. Blades show several views (Figure 31), of which the following are the most common: (*a*) parallel extinction, positive elongation, *O.A.* at edge of field (optic sign positive, $2V$ large), *O.A.P.* normal to long crystal axis, and (*b*) parallel extinction, negative elongation, interference figure indefinite (probably a slightly off-center Bx_a).

Mixed Fusion with Thymol. A mass of thin overlapping short blades grow into the thymol.

CLIONASTEROL

Cooling and Solidification of Melt. A fine-grained, gray, barely visible mesomorph spreads over the melt. The preparation solidifies to a glass, and it is impossible to cause crystallization by ordinary methods.

CLIONASTERYL ACETATE

Behavior on fusion is identical with that shown by chalinasteryl acetate. If the two compounds are compared with one another by means of a mixed fusion (*4*), it is readily seen that they form solid solutions, and are probably isomorphous.

DISCUSSION

This work clearly reveals both the strength and weakness of fusion analysis as a means of identification. The appearance of some fusions—for example, cholestane—is so remarkable that subsequent recognition is instantaneous, even if the observer is ignorant of optical crystallography. On the other hand, for compounds which decompose markedly at the melting point, the method is nearly useless; this will also be true if the compound does not crystallize—clionasterol, for example. Compounds which behave like clionasterol may sometimes be induced to crystallize by prolonged heating just below the melting point. Such treatment, however, requires apparatus not used in the present study. Fortunately, the formation of glasses which will not crystallize is rather rare. Isomorphism, as illustrated between the acetates of chalinasterol and clionasterol, may also cause difficulties. This again is a relatively rare occurrence; moreover, the fusion technique would identify the unknown as one of a very few possibilities.

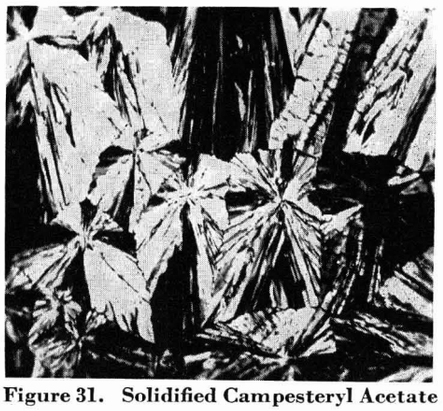

Figure 31. Solidified Campesteryl Acetate

The use of compiled fusion data to identify an unknown presents a small problem. In laboratories habitually concerned with relatively few compounds, there are no difficulties. The fusions of a few known compounds are easily memorized, and unknowns can then be recognized in a few seconds. If the unknown can be any one of hundreds, this method becomes impossible. Punched cards seem to offer the simplest way out of this difficulty. A laboratory could card index the gradually accumulating fusion data (*5*) in any one of a number of ways. The card for each compound should preferably have a photograph of the fusion attached. Identification of an unknown from information available in this form should be relatively rapid. A recent publication (*2*) has shown how similar data, for crystal optics, may be conveniently indexed on such cards.

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Determination of Lactose in Biological Materials

M. G. HOROWITZ, H. M. DAVIDSON, F. D. HOWARD, AND F. J. REITHEL
University of Oregon, Eugene, Ore.

The determination of lactose in biological materials is often difficult. The specific problem at hand was that of determining small changes in lactose concentration in metabolizing mammary gland homogenates. No method in the literature was sufficiently sensitive, specific, and convenient. Three methods were tested. A differential fermentation method employing yeasts was found useful as a check, but was too cumbersome for routine work. A manometric method involving the use of lactase was satisfactory if certain interfering substances were removed. The most rapid and convenient method is colorimetric. The lack of satisfactory methods for lactose analysis has impeded progress and cast doubt upon many investigations in the field of lactose metabolism. The present paper offers details and modifications of two methods previously used and one new method.

STUDIES in progress in this laboratory on the mechanism of lactose synthesis in mammary gland homogenates have posed problems in the determination of lactose. Others (*2*, *7*) have expressed the view that methods for determining this sugar are unsatisfactory when applied to complex mixtures. It is mandatory to have at hand a method which is specific, sensitive, and convenient if possible.

A review of the literature revealed a number of methods for determining lactose based on the following: reducing power before and after acid hydrolysis (*8*, *11*), manometric determination of galactose and/or glucose before and after hydrolysis (*14*), differential fermentation (*3*, *10*, *16*), paper chromatography (*5*), and color reaction with alkali and methylamine (*6*).

In the authors' hands, as in others' (*7*), the use of methods employing acid hydrolysis led to spurious results. Presumably polysaccharides of low molecular weight are present, which interfere. In homogenates containing only glucose and lactose it was found impractical to remove glucose by yeast adsorption (*4*) or fermentation and to determine lactose by the change in reducing power. Lactose seemed to interfere with the adsorption of glucose by yeast, and there were always present small but inconstant amounts of nonlactose reducing substances after fermentation. A further complication was introduced by the fact that the concentrations of lactose involved were so low as to dictate minimum dilution due to deproteinization procedures. Under such conditions all nonsugar reducing substances were difficult to remove from mammary gland preparations.

Paper chromatography was found to be very useful for qualitative explorations, but the quantitative methods were deemed too tedious and exacting in experiments where many determinations were to be made. Thus it seemed desirable to test and adapt other methods to the needs.

COLORIMETRIC METHOD

Principle. Compounds containing the 1,4 glucosidic linkage react with alkali and methylamine to yield a pink color (*1*). Recently it was shown (*6*) that this can be made the basis of a quantitative method. The following modification was found to be much more convenient and somewhat more sensitive.

Reagents. Methylamine hydrochloride, 1.6%. Sodium hydroxide, 5.4 *N*.

Procedure. To each of a series of Klett tubes was added 0.3 ml. of reagent made by adding 2 volumes of 1.6% methylamine hydrochloride to 1 volume of 5.4 *N* sodium hydroxide. The samples containing lactose were added and the volumes were adjusted to 5.0 ml. with distilled water. Each tube was fitted with a cork through which passed a section of 7-mm. outside diameter capillary tubing. Through the test solutions was bubbled a stream of nitrogen purified by passage over hot copper. At the end of 10 to 15 minutes the corks were firmly seated in the tubes, and the capillaries were withdrawn above the 5-ml. mark and closed off by means of a small clamp. A manifold was used, so that six or more tubes could be handled at one time.

The tubes were now heated at 55° in a serological water bath for 30 ± 0.5 minutes and cooled in tap water. Color development was estimated with a Klett-Summerson photoelectric colorimeter, using the 54 filter. Readings taken 15 minutes after withdrawal from the water bath were nearly identical with those made at 2 minutes.

A typical standard curve is illustrated by the values in Table I. Such values were found to be highly reproducible.

Recovery of lactose from homogenates was excellent if the following interfering substances, when present, were removed: monosaccharides, maltose, cellobiose, sugar phosphates, and calcium and barium ions.

Table 1. Determination of Lactose

Lactose, Mg.	Klett Reading
0.25	10
0.50	32
1.00	87
2.00	194
3.00	288

MANOMETRIC METHOD

Principle. In developing this method an attempt was made to circumvent the difficulties inherent in accurately determining true sugar reducing power and to avoid dilution attendant upon deproteinization. Winzler (*17*) has shown that it is possible to determine glucose manometrically by yeast fermentation in the presence of sodium azide. This procedure was used to determine the glucose produced when lactose was hydrolyzed in the presence of lactase.

In order to achieve complete hydrolysis it was found imperative to use a lactase-lactose ratio of about 0.5, maintain a pH of 6.8 during incubation, and remove oxygen from the solutions and incubate in the absence of oxygen.

Apparatus and Reagents. A Warburg respirometer was used to measure the carbon dioxide evolved during fermentation.

Sodium succinate buffer, 0.15 *M*, pH 4.5.

Sodium azide, 0.06 *M*.

A suspension of baker's yeast washed five times with distilled water, 75 mg. per ml.

Lactase A concentrate (Rohm & Haas).

Procedure. A 15.0-ml. sample of a 10% mammary gland homogenate was centrifuged and the supernatant was aerated with nitrogen for several minutes. Three 4.0-ml. aliquots were taken; one served as a blank, to the second were added 20 mg. of enzyme, and to the third were added 20 mg. of enzyme and 5.0 mg. of lactose. After dilution to 8.0 ml. each solution was adjusted to pH 6.8. After incubation for 1 hour at 37° 0.5-ml. aliquots were taken for analysis.

Table II. Determination of Lactose in Mammary Gland Homogenates

Homogenate Analyzed, Ml.	Lactose Added, Mg.	Lactose, Mg./G. Tissue	Lactose Recovered, Mg.
0.50	...	17.3	...
0.50	...	11.24	...
0.50	0.50	...	0.55
0.50	0.50	...	0.45
0.50	0.50	...	0.50
0.50	0.50	...	0.50

Each series of determinations included: yeast blank, glucose standard, lactase blank, homogenate plus lactase (basal lactose value), and homogenate and lactase and added lactose (recovery).

Into the body of a Warburg flask were introduced 1.0 ml. of sodium succinate buffer, 1.0 ml. of sodium azide, and the sample to be analyzed. A total volume of 3.0 ml. was used. Into the side arm was pipetted 0.50 ml. of washed yeast suspension. After temperature and nitrogen equilibration the organisms were tipped into the body of the flask and readings were taken until carbon dioxide evolution ceased, usually about 25 minutes. Characteristic results are shown in Table II.

Several sugars were tested for interference. Experiments similar to those above were set up with test solutions containing sugars at about the same concentration and in addition to lactose. Lactose recovery in the presence of sucrose and maltose was near 100%, but low recoveries were found in the presence of melibiose, glycogen, xylose, and cellobiose. This phenomenon, to be described more fully in another communication, is due to the effect of these sugars on yeast in the presence of azide and is not due to inhibition of lactase action.

DIFFERENTIAL FERMENTATION METHOD

Principle. This method is based upon the observation that *S. bayanus* (NRRL 966) fermented only glucose, *S. carlsbergensis* (NRRL 379) fermented glucose and galactose in a mixture of these two sugars with lactose, and *S. fragilis* fermented all three. Reducing power was determined before and after fermentation by each organism.

Reagents. Actively growing broth cultures of the yeasts were centrifuged, washed three times with distilled water, and suspended in enough water to give a reading of 600 to 800 on the Klett-Summerson photoelectric colorimeter, using filter No. 54.

To determine reducing power, Somogyi's copper reagent (*12*) was used together with Nelson's chromogenic reagent (*9*). When necessary, samples were deproteinized with zinc sulfate and barium hydroxide as described by Somogyi (*13*).

Procedure. The fermentation of the samples to be analyzed was conducted in graduated centrifuge tubes. If more than 5 mg. of sugar remained after fermentation, or more than 15 mg. were initially present, 50-ml. tubes were used. To these tubes were added 3 ml. of yeast extract solution, the sample, and enough water to bring the volume to 8 ml. If smaller samples were analyzed, fermentation was carried out in 15-ml. tubes to which were added 1.5 ml. of yeast extract, the sample, and water to a volume of 5 ml. Such tubes were plugged with cotton and sterilized for 5 minutes at 15 pounds' pressure. The cooled tubes were inoculated with 2 ml. of the appropriate yeast suspension and incubated.

The inoculum for samples from which lactose and one or both of the other sugars were to be removed consisted of 1 ml. of *S. fragilis* suspension and 1 ml. of *S. carlsbergensis* suspension. During 48-hour incubation quantitative removal of amounts of sugars up to 20 mg. was effected. Two-milliliter inocula of *S. carlsbergensis* removed similar amounts of glucose and galactose in 48 hours. Similar inocula of *S. bayanus* were found to remove glucose quantitatively in 3 to 4 hours, but began to adapt to galactose if incubated for more than 36 hours. A great many control experiments showed that small quantities of the three sugars were fermented completely under the conditions described and that only those fermentations stated above for each yeast actually occurred.

As an example, the analysis of a sample containing 80.2 mg. of glucose, 52.9 mg. of galactose, and 131.8 mg. of lactose in 50 ml. is described in detail.

A 1-ml. aliquot of the original solution (Solution A) was diluted to 50 ml. A 1.00-ml. aliquot of the dilute solution was found to give a reading of 110.5 units. The units referred to are the scale readings on the Klett-Summerson photoelectric colorimeter which are designated as optical density × 1000. The total reducing power of Solution A was therefore 110.5 × 50 × 50 = 276,000 units. Reducing equivalents of 0.1 mg. of standard sugars were determined simultaneously and found to be: glucose, 133.5 units; galactose, 106.5; lactose, 79.0.

A fermentation series was set up in duplicate with each yeast as previously described. Five milliliter aliquots of Solution A were used. Yeast blanks included in the set differed only in that they contained no sugar. After incubation, the samples were diluted to 40- to 50-ml. volume (accurately estimated) and the yeasts were centrifuged down. Aliquots of the supernatant were then filtered through Whatman No. 50 filter paper until clear. A 0.5- to 1.0-ml. aliquot was used to determine the reducing power.

In each group of tubes heated were the unknowns, the appropriate yeast blanks, the copper reagent blanks, and the appropriate standard sugars. For best results fermentations were conducted in duplicate and reductions were run on each sample in triplicate.

The samples fermented by *S. fragilis* and *S. carlsbergensis* had been diluted to 40.0 ml. Aliquots of 0.8 ml. showed an average reducing power (after subtraction of the blanks) of 9.5 units. 9.5 × 500 (dilution) = 4750 units of total residual reducing power for Solution A.

The samples fermented by *S. carlsbergensis* had been diluted to 50.0 ml. Aliquots of 1.00 ml. showed an average reducing power (corrected) of 236 units. 236 × 500 (dilution) = 118,000 units, which represented reducing power due to lactose plus residual substances. 118,000 − 4750 = 113,000 units due to lactose. The reducing equivalent for lactose in this heating run was 830 units per mg. 113,000 ÷ 830 = 136.3 mg. of lactose.

The samples fermented by *S. bayanus* had been diluted to 50.0 ml. Aliquots of 0.50 ml. showed an average reducing power (corrected) of 177.0 units. 177.0 × 1000 (dilution) = 177,000 units which represented lactose, galactose, and residual substances. In this heating run lactose exhibited a reducing equivalent of 845 units per mg. and therefore 136.3 mg. × 845 units per mg. = 115,000 units were due to lactose. 177,000 − 115,000 = 62,000 units. This minus 4750 (residual) = 57,000 units due to galactose. In this heating run galactose exhibited a reducing equivalent of 1105 units per mg. and therefore 57,000 ÷ 1105 = 51.6 mg. of galactose.

Table III. Analysis of Sugar Mixtures

Glucose, Mg.		Galactose, Mg.		Lactose, Mg.	
Initial	Found	Initial	Found	Initial	Found
91.3	83.3	..	..	205.3	213.0
83.3	81.3	...	...	101.8	99.7
51.0	54.2	78.3	71.0	...	...
80.2	81.3	52.9	51.6	131.8	136.3

The glucose content was determined by difference. 136.3 mg. of lactose × 790 (the reduction equivalent in the initial determination of total reducing power) = 107,500 units. 51.6 mg. of galactose × 1065 (reduction equivalent in initial heating run) = 55,000. Thus the total reducing power, 276,000 minus 107,500, minus 55,000, minus 4750 (residual) = 108,500 units due to glucose. This divided by the glucose equivalent of the initial run, 1335 units per mg. = 81.3 mg. of glucose.

The above results, plus additional analytical data, are summarized in Table III.

It is difficult to assign percentage accuracy, because a great many analyses showed that this quantity varied markedly with the absolute and relative levels of the sugars present. Average deviations were of the order of 3%. However, in several tissue analyses where, for example, a 4-ml. sample contained 50 mg. of glucose and 5 mg. of lactose, the former sugar could be estimated within 2%, while results for the latter were in error by as much as

8%. The lower limit of reliability was found at a concentration of about 8 mg. of lactose per 100 ml. of protein-free filtrate.

The procedure developed for pure sugar solutions was applied directly to zinc sulfate–barium hydroxide filtrates of mammary gland homogenates. Among the substances that were frequently added to homogenates such as adenosine triphosphate, diphosphopyridine nucleotide, magnesium chloride, succinate, phosphate, fructose diphosphate, and creatine, only the last compound was found to interfere with the sugar determinations.

When galactose was not added the analysis was somewhat simplified. No galactose could be detected in lactating tissue and the glucose content was also very low.

Although the glucose content was generally determined by difference, it was found possible to obtain an independent check by the use of the Tauber-Kleiner method (*15*).

SUMMARY

Each of the methods investigated is applicable to the determination of lactose in mammary homogenates, and presumably other similar materials, when glucose and galactose are the only other sugars present. Each substrate or coenzyme added had to be treated as a separate variable and appropriate measures taken to remove each interfering substance not normally present in mammary gland. When lactose was determined in aliquots of the same sample with each method, the same result was obtained within the limits of experimental error.

The colorimetric method is by far the most convenient, but care must be taken markedly to lower the concentration of reducing sugars other than lactose, to remove sugar phosphates, and to remove calcium and barium ions.

The use of lactase has been found very convenient for analyses where only glucose, galactose, and lactose are present in tissue preparations.

The differential fermentation method is much more cumbersome and time-consuming but is relatively insensitive to other sugars or to sugar phosphates. Its chief value is that of a confirming method.

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NOTES ON ANALYTICAL PROCEDURES . . .

Determination of Oxygen in Zirconium Metal by the Vacuum Fusion Method

J. K. STANLEY, JOAN VON HOENE, AND GEORGE WIENER
Westinghouse Research Laboratories, East Pittsburgh, Pa.

TWO methods are generally used for determining the oxygen content of metals: residue methods in which the metals are removed by some chemical action, leaving unattacked oxides, carbides, nitrides, etc., which can then be further analyzed and the oxygen estimated; and reduction methods in which the oxides are generally reduced by the action of carbon, but sometimes by hydrogen, and the gases formed are analyzed. These methods determine only the total oxygen.

To date only the residue methods have been used for determining oxygen in zirconium metal. Lilliendahl, Wroughton, and Gregory (*4*) discuss two methods. One method consists of completely burning the metal to oxide and calculating the oxygen from the increase in weight. Obviously, one must have a complete analysis of the sample for metallics, including hafnium, and nonmetallics, such as carbon and nitrogen. The other method consists of reacting the zirconium with chlorine and vaporizing the zirconium tetrachloride. The oxide, carbide, and possibly nitride, then remain. The carbon and nitrogen contents must be known.

Read and Zopatti (*7*) have devised a method of determining oxygen, which is based on the fact that zirconium and most of the metallic impurities may be volatilized as chlorides upon treatment with dry hydrogen chloride gas at temperatures as low as 450° C.

The residue methods generally require long times (hours) to make a single analysis; the procedures are tedious because of the many manipulations, and in many cases the results are open to various uncertainties (*1–9*). In view of these considerations, it seemed desirable to consider the well developed vacuum fusion method for determining oxygen in metals. This method is rapid; the analyses require 15 to 30 minutes for a single determination. The procedure is relatively simple and the analysis is more positive; the presence of other metals, oxides, carbides, nitrides, etc., does not interfere.

VACUUM FUSION METHOD

The vacuum fusion method depends upon the reduction of the oxides by carbon in the fluid metal; the carbon monoxide formed is converted into carbon dioxide and this is then measured in terms of its pressure exerted in a calibrated volume. The

apparatus used has been described by McGeary, Stanley, and Yensen (*5*). The vacuum-fusion method was first devised for determining oxygen in ferrous metals, but can be used for nonferrous metals as well. The determination of oxygen in nonferrous metals is generally done in a molten iron bath contained in a graphite crucible. In this way, the iron is saturated with carbon. The addition of a metal such as zirconium gives a dilute solution of zirconium in iron. The zirconium oxide can then react with the carbon:

$$ZrO_2 + 3C \longrightarrow ZrC + 2CO$$

When the present work was undertaken, there was some doubt as to the applicability of the method to the determination of oxygen in zirconium. However, Prescott (*6*) and more recently Kroll and Schlechton (*3*) have shown that reduction of zirconium oxide with carbon can take place in vacuum. Walter (*9*) and Derge (*1*) have demonstrated successful methods for the determination of oxygen in titanium by vacuum fusion. Similarity of zirconium oxide and titanium oxide can be expected on the basis of the positions of titanium and zirconium in the periodic table and from thermodynamic data.

The free energy and equilibrium constant for both the reduction of titanium oxide and zirconium oxide with carbon were calculated and indicate that the reduction of the oxides is possible.

Consider the reaction:

$$TiO_2(s) + 3C(\text{graphite}) \longrightarrow 2CO(g) + TiC(s)$$
$$K_A = (P_{CO})^2 \qquad (A)$$

The free energy, ΔF_A, at 2173° K., is calculated to be −73,675 calories. (The temperature of 1900° C. was chosen for the reaction because it gave more reliable results than lower temperatures.) In making this calculation a linear extrapolation of the data for titanium carbide and titanium oxide had to be made from 1800° to 2173° K. Inasmuch as no transitions occur, this was believed to introduce only a minor error. The equivalent equilibrium constant, K_A, is 2.82×10^7 at this temperature.

Consider the reaction:

$$ZrO_2(s) + 3C(\text{graphite}) \longrightarrow 2CO(g) + ZrC(s)$$
$$K_B = (P_{CO})^2 \qquad (B)$$

The free energy, ΔF_B, at 2173° K. is calculated to be −19,200 calories, and an equilibrium constant, K_B, equals 87. The values for ΔF_B and K_B were calculated from the data of Prescott (*6*), which involved an extrapolation from 2015° to 2173° K.

Table I. Typical Results

Sample	Temperature °C.	Sn	Reaction Time[a] Min.	Sample Weight Gram	Oxygen by Vacuum Fusion %	Approximate Oxygen Content[b] %
1	1850/1900	...	10	0.097	0.205	0.16D
		...	10	0.091	0.215	0.16
		...	10	0.087	0.225	0.16
		Added	10	0.102	0.240	0.16
		Added	10	0.078	0.236	0.16
		Added	10	0.055	0.226	0.16
2	1050/1900	...	10	0.072	0.099	0.089D
		...	10	0.092	0.123	0.089
		Added	10	0.073	0.179	0.089
		Added	10	0.078	0.172	0.089
3	1900	...	10	0.098	0.124	0.13C
		Added	10	0.090	0.133	0.13
4	1850/1900	Added	10	0.078	0.286	0.17D
		Added	10	0.127	0.220	0.17
5	1900	...	10	0.080	0.282	0.32C
		...	10	0.065	0.292	0.32
		Added	10	0.113	0.325	0.32

[a] Includes time at which sample was dropped to time reaction was discontinued—i.e., when gas evolution ceased as measured by thermocouple vacuum gage.

[b] Analyses run by chlorine volatilization method (C) discussed by Lilliendahl, Wroughton, and Gregory (*4*), or were doped samples (D)—i.e., samples to which oxygen has been added deliberately and oxygen estimated by weighing.

Because no data are available for zirconium carbide, no independent check of the experimental results can be made. Subsequent experimental results given below, and the methods of Walter and Derge, qualitatively bear out the thermodynamic calculations, which indicate that both reactions are strongly favored to proceed as written.

Procedure. The vacuum fusion apparatus, its manipulation, and a survey of the results obtainable have been presented (*5*). However, certain modifications had to be introduced into the procedure before reliable and consistent results were possible on zirconium. This discussion therefore is concerned with the modifications which were necessary to get reliable data.

The three modifications made in the procedure were:

1. The addition of the zirconium sample must result in a dilute solution of zirconium in the iron. The required dilution can be obtained by using small samples (0.05 to 0.10 gram) and introducing about 15 to 20 grams of iron prior to the first zirconium samples. Thereafter about 1.5 grams of iron should be added before each sample is analyzed. Improper dilution of the zirconium with iron results in obtaining less and less oxygen on subsequent samples.
2. The reaction temperature should be between 1850° and 1950° C., preferably on the higher side for consistent results. Temperatures as low as 1650° C. can be used, but in general the results will be on the low side.
3. The addition of tin in amounts up to 25% appears to facilitate the reduction of zirconium oxide. Additional amounts up to 50% tin did not noticeably affect the procedure. It may be argued that increasing the heating time per sample would give results comparable to those obtained by the use of tin. However, experiments with longer heating periods than 10 minutes failed to show any measurable increase in the oxygen content.

The exact role of tin in this connection is not known. Originally Reeve (*8*) added tin as a flux to lower the melting point of his charge, but in the authors' case the operations are carried out at about 1900° C., so that the tin in some way may affect the fluidity of the molten charge, which may be conducive to more rapid reaction of the carbon with zirconium oxide.

PREPARATION OF SAMPLES

The work reported here was carried out with small samples cut from large pieces of zirconium. As the success of the analysis depends upon the proper dilution of the zirconium sample in a mixture of iron and tin, samples of the order of 0.1 gram were used, because the apparatus could not easily be adapted to use greater quantities of iron and tin. The use of such small samples is no liability if the oxygen is uniformly distributed. If the oxygen were heterogeneously distributed in a large section, samples of 1 to 5 grams would hardly be representative. The nature of the sample touches on the whole philosophy of sampling. The best one can do is to take a sufficient number of samples, regardless of size, until a representative average value of oxygen in a section is obtained.

The samples used were cut to size with a hacksaw and burrs were filed off, as they have a high oxygen content. After cutting and filing, the sample was washed in benzene and acetone. If necessary after cutting, the sample was etched in dilute hydrofluoric acid and washed in acetone.

One precaution had to be observed in these analyses. Invariably, if the zirconium sample came in touch with the graphite crucible at about 1900° C., as it was introduced into the crucible for analysis, it immediately wetted the crucible above the molten iron and no oxygen analysis could be obtained. In order to avoid this difficulty, samples were wrapped with annealed iron foil approximately 0.5 × 0.5 inch (1.25 × 1.25 cm.) (SAE 1010 steel, 0.0015 inch thick) and weighing about 0.080 gram. The wrapped sample was then washed in benzene and acetone and introduced into the molten iron without difficulty, and reliable results were obtained. Oxygen due to the steel foil analyzed at 0.025% was equivalent to 0.002% on a 1-gram sample and this was subtracted with the blank, which averaged about 0.004%

oxygen, based on a 1-gram sample, for a 10-minute analysis. This is higher than normal blanks (0.0005 to 0.001%), but could be decreased with longer degassing time. (Four hours at 2000° C. were used in this work.) However, the blank as found is accurate enough for most samples with high oxygen content. Oxygen content of the tin used for dilution was found to be 0.007%.

RESULTS

Some typical results and operating conditions are given in Table I. The results approximate the oxygen suspected in the samples prepared by deliberate additions of oxygen or by Lilliendahl's chlorine method (*4*). The oxygen determined by vacuum fusion is higher than that estimated by doping—i.e., oxygen is added deliberately by oxidation and the weight increase is determined; this might be expected if the oxygen content of the starting material before doping is not accurately known. The agreement between the vacuum fusion method and the chlorine method is reasonably good.

The vacuum fusion method is very rapid, as it requires only about 20 minutes per sample.

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Effect of Temperature on Density and Refractive Index on Organic Compounds of Various Cox Chart Families

R. R. DREISBACH
The Dow Chemical Co., Midland, Mich.

IN THE author's paper on the Eykman equation (*1*), it was demonstrated that this empirical equation relates liquid density and refractive index accurately for a narrow temperature range around room temperature. Kurtz, Amon, and Sankin (*4*) demonstrated that this equation was applicable over a wide range of temperature. Ward and Kurtz (*5, 6*) recommended that the empirical equation, $\Delta n = 0.6\,\Delta d$, was accurate for hydrocarbons for small changes of temperature, and a column in the tables (*4*) demonstrated the variation of the value of n_D calculated by this formula from determined values.

Griswold (*3*) differentiated the Eykman equation:

$$\frac{(n^2 - 1)}{(n + 0.4)} \times \frac{1}{d} = C \qquad (1)$$

and obtained:

$$\Delta n/\Delta d = dn/dd = \frac{C(n + 0.4)^2}{(n + 0.4)^2 + 0.84} \qquad (2)$$

Griswold (*3*) demonstrated that the coefficient 0.6 was good for hydrocarbons where the C value was 0.74 or greater, but that where the C value was low, as in the case of nonhydrocarbons, the coefficient of proportionality between n and d might be as low as 0.3.

Dreisbach and Martin tabulated the C values for 98 organic compounds in 15 Cox chart families. This C value varies from 0.80806 for *m*-divinylbenzene to 0.31522 for 1,2,3-tribromobutane. This coefficient is in all cases very close to 0.80 times the C value and, hence, the variation of refractive index with density can be represented by:

$$\Delta n/\Delta d = 0.8C \qquad (3)$$

The relationship of Equation 3 holds at temperatures of 10° C. up to 50° C., at least in every case tested.

Table I records the C value, the refractive index at 20°C. (n_D^{20}) from (*1, 2*), the dn/dd values calculated by means of Equation 2 and the ratio $(dn/dd)/C$. In every case the ratio of Equation 2 lies between 0.79 and 0.82, except in the case of *o*-dibromobenzene, where it is 0.83. When the coefficient 0.80 in Equation 3 is replaced by 0.79 in one case and 0.82 in the other, it is found that the difference between the two values of Δn is 0.0001 and, hence, when the value 0.80 is used the error in Δn would be less than 0.0001.

Table I. Refractive Index at 20° C., C Value of Eykman Equation, Variation of Refractive Index with Density, and Ratio of Variation with C Value

Compound	n_D^{20}	C	dn/dd	$(1/C) \times (dn/dd)$
Hexane[a]	1.37500	0.76093	0.600	0.79
Benzene[a]	1.50110	0.75000	0.608	0.81
Chlorobenzene	1.52406	0.62170	0.510	0.82
o-Dichlorobenzene	1.55145	0.55211	0.452	0.82
Bromobenzene	1.55972	0.48900	0.401	0.82
o-Dibromobenzene	1.51101	0.40000	0.331	0.83
1-Ethyl-4-vinylbenzene	1.53763	0.78880	0.644	0.82
1-Bromo-3-vinylbenzene	1.59268	0.54459	0.449	0.82
Divinylbenzene	1.57610	0.80806	0.665	0.82
Methyl ethyl ketone	1.37850	0.60854	0.481	0.79
n-Octyl alcohol	1.42913	0.69036	0.552	0.80
Acetic acid	1.37160	0.47428	0.374	0.79
Ethyl acetate	1.37239	0.55346	0.436	0.79
Nitropropane	1.40161	0.53488	0.425	0.79
Nitrotoluene	1.54662	0.61809	0.506	0.82
n-Amyl ether	1.41195	0.70009	0.557	0.79
Phenetole	1.50735	0.69101	0.561	0.81
p-Chlorophenetole	1.52521	0.61209	0.499	0.81
Propionphenone	1.52684	0.68425	0.558	0.81
Propionitrile	1.36635	0.61778	0.495	0.79
β-Phenylethyl alcohol	1.53252	0.68402	0.558	0.81
Phenol	1.54178	0.67055	0.548	0.82
Aniline	1.58545	0.74616	0.615	0.82
n-Butyl chloride	1.40211	0.60481	0.480	0.79
1,2-Dichloropropane	1.43901	0.50377	0.404	0.80
Perchloroethylene	1.50534	0.40947	0.329	0.80
1,2-Dibromoethane	1.53865	0.32366	0.264	0.81
1,2,3-Tribromoethane	1.58597	0.31522	0.260	0.82
Carbon tetrachloride	1.46005	0.38171	0.307	0.80

[a] Values from (*1*); all the rest from (*2*).

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Stannous Chloride–Iodine and Zinc-Ferrocyanide Titrations

Application of the Dead-Stop End Point

D. F. SWINEHART

University of Oregon, Eugene, Ore.

THE dead-stop end-point apparatus of Foulk and Bawden (*6*), which they applied to the iodine-thiosulfate and the iodine-arsenite titrations, has been found to be applicable to several other titrations (*1–4, 8*). In this paper the characteristics of the end points for the stannous chloride–iodine and the zinc-ferrocyanide titrations using this apparatus are described.

STANNOUS CHLORIDE–IODINE TITRATION

A dilute solution of stannous chloride in 0.1 *M* hydrochloric acid may be titrated with a dilute iodine solution, using the dead-stop apparatus to detect the end point, if proper precautions are taken to eliminate atmospheric oxygen from the solutions. Not only does the presence of oxygen introduce error due to the oxidation of stannous ion, but the end point does not function properly in the presence of oxygen. Ten-milliliter titrations of 0.005 *N* stannous chloride with 0.005 *N* iodine were successfully carried out by working in an atmosphere of carbon dioxide or purified nitrogen. The stannous chloride solution was made up in a three-necked flask and the inert gas was bubbled through it for about 2 hours before use. This time was required to stabilize the solution so that the normality did not change at an appreciable rate. It was found that carbon dioxide directly from the tank was much better than nitrogen, even after the latter was purified by bubbling through alkaline pyrogallol or Fieser's solution (*5*). Using nitrogen, the stannous chloride solution never became stable, but the normality changed at the rate of 2 to 3% per hour even after long bubbling. After 2 hours' bubbling with carbon dioxide the solution became so stable that, with continuous bubbling, the normality did not change appreciably in a day or two.

A sample for titration was removed by pipet and the titration was carried out in a large test tube while the solution was stirred with a stream of carbon dioxide. The burets had fine capillary tips which were immersed in the solution during titration.

With excess stannous chloride in the titration vessel and 50 mv. across the electrodes, the galvanometer (sensitivity 1.7×10^{-8} ampere per mm.) showed its characteristic small, steady deflection. As iodine was added, near the end point, the galvanometer showed small deflections, returning each time to its steady reading with stirring. A permanent additional deflection indicated the end point. The apparatus thus behaves as it does for the thiosulfate-iodine end point (*6*).

Duplicate 10-ml. titrations agreed to a precision of 1 to 2 parts per thousand.

ZINC-FERROCYANIDE TITRATION

The apparatus responds in a similar manner to the titration of zinc ion in dilute sulfuric acid and ammonium sulfate at 60° to 80° C. A potential of 200 mv. was used across the dead-stop electrodes. Less than 150 mv. resulted in a less satisfactory and less sensitive end point. If the solution contains excess zinc, the galvanometer shows its characteristic small deflection and a sharp additional deflection occurs when a trace of excess ferrocyanide is added. The presence of ammonium sulfate was found to be necessary in order to get a sharp deflection at the end point. The reason for this is not apparent. Zinc solutions containing 2 grams of ammonium sulfate and 2 ml. of 6 *N* sulfuric acid in 100 ml. of solution behaved satisfactorily.

Using a galvanometer with a sensitivity of 1.7×10^{-8} ampere per mm., the end point of the titration of 0.01 *M* zinc sulfate with 0.0067 *M* ferrocyanide is sharp. It is possible to dilute both solutions yet another factor of 2 and still obtain a satisfactory end point. In general, these titrations were reproducible to 1 to 2 parts per thousand. Representative results are shown in Table I. The precision of results was about the same at all dilutions down to 0.005 *M* zinc sulfate and 0.0033 *M* ferrocyanide.

Several hundred zinc analyses have been carried out in this laboratory using 0.0067 *M* ferrocyanide with consistently excellent results. The titration is rapid and no delay is involved except for the time required to heat the solutions to about 70° C. The end point is sluggish below 60° C.

Table I. Representative Results

(Galvanometer sensitivity 1.7×10^{-8} ampere per mm. Potential across dead-stop electrodes 200 mv. Concentrations are approximate only)

0.01 *M* $ZnSO_4$, Ml.	0.0067 *M* $K_4Fe(CN)_6$, Ml.	Ratio
5.00	5.04	1.008
5.00	5.03	1.006
10.00	10.07	1.007
10.00	10.09	1.009
15.00	15.10	1.007
15.00	15.07	1.005
20.00	20.16	1.008
20.00	20.15	1.008

Av. ratio, 1.007.
Av. deviation from mean, 1 part per thousand.

Attempts to titrate 0.001 *M* zinc sulfate with 0.00067 *M* ferrocyanide using a galvanometer with a sensitivity of 3×10^{-9} ampere per mm. were less successful. The end point is no longer sharp at these dilutions. The galvanometer deflection sets in gradually, presumably because of the finite solubility of the slightly soluble salt, $K_2Zn_3[Fe(CN)_6]_2$. Titrations can be carried out with these solutions with somewhat less precision (3 to 4 parts per thousand) by titrating to an arbitrarily chosen deflection.

Using a meter of sensitivity 1 to 3×10^{-6} ampere per mm. instead of the galvanometer, 0.1 *M* zinc sulfate may be titrated with 0.067 *M* ferrocyanide. This has the advantage of great simplicity when a relatively inexpensive and rugged meter is used. It has obvious advantages over the use of an external indicator for the detection of the end point. It is easily as good as the end point using sodium diphenylamine sulfonate, and it does not involve the visual judgment of a color change.

Because the stoichiometry of the reaction of zinc with ferrocyanide has been the subject of previous investigations—e.g., that by Kolthoff and Pearson (*7*)—this subject has not been investigated here. The advice of these authors that the conditions must be maintained constant if reproducible results are desired was followed. Ammonium sulfate was added to the titration mixture because they advise it, and was later found to be a necessary component. No study was made of interferences. Presumably the same interferences may be expected with this method as with other methods of detecting the end point. Reagent grade chemicals and conductivity water were used throughout the work.

ACKNOWLEDGMENT

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Tetrahydroxy Cobalt(II) Ion as a Qualitative Test for Cobalt

SAUL GORDON AND JAMES M. SCHREYER
University of Kentucky, Lexington, Ky.

WHEN a cobaltous salt solution is added to an excess of saturated sodium or potassium hydroxide solution, the cobaltous hydroxide that precipitates locally dissolves upon stirring to produce a deep blue solution which contains tetrahydroxy cobalt(II) ion (*5*). In the course of studying the spectrophotometric properties of this cobaltous complex in strongly alkaline solutions, the authors realized that the blue solution formed is specific enough to be utilized as a confirmatory test for the qualitative analysis for the cobaltous ion. The formation of this complex ion may be represented by the following equations:

$$Co(H_2O)_6^{++} + 2OH^- \longrightarrow Co(OH)_2(H_2O)_4\downarrow + 2H_2O$$

$$Co(OH)_2(H_2O)_4 + 2OH^- \longrightarrow Co(OH)_4(H_2O)_2^{--} + 2H_2O$$

Reichel (*4*) suggested the use of the slight solubility of cobaltous hydroxide in concentrated alkalies for the qualitative separation of small amounts of cobalt from nickel, the latter forming an insoluble hydroxide. Donath (*2*) investigated some of the chemical properties of this complex, postulating the presence of a cobaltite ion, CoO_2^{--}. Alvarez (*1*) reported that 1 drop of a 1% cobalt salt solution produces a pronounced blue color when added to a boiling solution of potassium or sodium carbonate. Qualitative reagents used as tests for cobalt were reviewed by the International Commission for Reactions and Reagents (*6*), but the application of this colored complex of cobalt was not reported. Although this reaction has been known for a long time, it has not been modified for adaptation to any scheme of cation analysis, nor have its limitations of detection and sensitivity been determined.

EXPERIMENTAL

The schemes most frequently used separate cobalt, nickel, and very often zinc, as a subgroup of the Group III cations. Therefore, Hogness and Johnson's (*3*) method of analysis for the zinc subgroup was modified to include this test for the detection of cobaltous ion. To determine the usefulness of this test, the Group III cations—aluminum, chromium, cobalt, iron, manganese, nickel, and zinc—were determined in many combinations on a semimicro scale. The test solutions varied in concentration from 0.3 to 50 mg. of the metal ions.

After dissolving the cobalt, nickel, and zinc sulfides in 1 ml. of 6 *M* hydrochloric acid and 1 ml. of 6 *M* nitric acid, heat the mixture to boiling, and then transfer it to a casserole. With a stirring rod coalesce the sulfur formed, remove, and discard it. Then evaporate the solution to dryness. Dissolve the residue in a buffer solution of 1 ml. of 2 *M* sodium bisulfate and 2 ml. of saturated sodium sulfate solution. Divide this solution into two equal portions, A and B. To portion A add 5 drops of 3 *M* ammonium acetate, and pass hydrogen sulfide into the cold solution for at least 1 minute. Heat in a low flame, gradually raising the temperature almost to boiling. The appearance of a white or very light gray precipitate of zinc sulfide indicates the presence of zinc. Evaporate portion B of the buffer solution in a casserole, almost to dryness. Cool and add 6 drops of water, dissolving as much solid as possible. Label this solution C. Add 4 drops of solution C to 1.5 ml. of saturated sodium hydroxide solution in a 4-ml. test tube. The formation of a blue precipitate at the top of the solution indicates the presence of cobalt. Tap the tube gently to allow the solutions and precipitate to mix. Centrifuge. A blue supernatant, especially near the upper surface of the solution (and usually a blue-tinged precipitate) confirm the presence of cobalt. This blue test for cobalt is best seen if compared with an equal amount of water against a white background. To 1 or 2 drops of the buffer solution C, add 1 drop of 15 *M* ammonia solution, 4 drops of water, and 2 drops of dimethylglyoxime reagent. The formation of a red precipitate indicates the presence of nickel.

DISCUSSION

Any white precipitate formed when the buffer solution is added to the saturated sodium hydroxide solution is sodium sulfate. A light green precipitate which might appear at this point is nickel hydroxide. The blue precipitate formed is either a basic cobaltous salt or white sodium sulfate colored by the cobaltous hydroxy complex ion. When the mixture is centrifuged the blue complex solution is readily discernible. The absence of a blue solution or a blue-tinged precipitate indicates the absence of cobalt, within the limits of sensitivity of the test. The reactions that take place when the buffered solution is added to the saturated sodium hydroxide solution, assuming that cobalt, nickel, and zinc are present, may be represented by the following equations:

$$Co(H_2O)_6^{++} + 2OH^- \longrightarrow Co(OH)_2(H_2O)_4\downarrow + 2H_2O \text{ (pink)}$$

$$Co(OH)_2(H_2O)_4 + 2OH^- \longrightarrow Co(OH)_4(H_2O)_2^{--} + 2H_2O \text{ (deep blue)}$$

$$Ni(H_2O)_6^{++} + 2OH^- \longrightarrow Ni(OH)_2(H_2O)_4\downarrow + 2H_2O \text{ (light green)}$$

$$Zn(H_2O)_4^{++} + 3OH^- \longrightarrow H[Zn(OH)_4]^- + 3H_2O \text{ (colorless)}$$

$$2Na^+ + HSO_4^- + OH^- \longrightarrow Na_2SO_4\downarrow + H_2O \text{ (white)}$$

This blue complex ion of cobalt may be formed in either saturated sodium hydroxide or saturated potassium hydroxide solutions. The saturated sodium hydroxide solution is more satisfactory because of the higher hydroxyl ion concentration, which produces a deeper blue color with the cobaltous ion. If the test is applied to a solution of cobalt without using the buffer solution, the sensitivity is about the same. The advantage of using the evaporated buffer solution is that the white precipitate of sodium sulfate aids in the centrifugation of any other precipitate present and accentuates the blue color of the complex of cobalt. Although this method of cobaltous ion detection is suitable for use on both semimicro and macro scales, it is not satisfactory as a spot test. For most satisfactory results with this cobalt test, the hydroxyl ion concentration must be greater than 12 *M*, which is not practical in spot test analyses. The test gave satisfactory results in the hands of a class of 126 in elementary qualitative analysis; 95.3 and 98.4% of them reported successful tests on their known and unknown Group III solutions, respectively.

SENSITIVITY

The smallest amount of cobalt which may be detected is about 0.05 mg. in 1 drop of solution added to 1.5 ml. of saturated sodium

hydroxide solution. This quantity is well within the amounts used in elementary qualitative analysis.

In addition, it is found that 1 mg. of cobalt gives an excellent test in the presence of 15 mg. of nickel; 5 mg. of cobalt and 50 mg. of nickel cause the formation of a blue-tinged light-green precipitate when analyzed by the above procedure. In the cases of high concentrations of nickel as compared to cobalt, the test is best made by comparing the color of the precipitate with the color of nickel hydroxide free of cobalt, formed with the same reagents.

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Dark-Chamber Titrimeter for Chemiluminescent Indicator Titrations in Colored Solutions

FREDERIC KENNY AND R. B. KURTZ

Hunter College, New York, N. Y.

THE instrument described obviates the use of a dark room in titrations employing a chemiluminescent indicator. It can be used in a brightly lighted room.

Ferrous solutions, highly colored with sufficient chromic ion to prevent the use of the usual redox indicators, were titrated with ceric sulfate, using siloxene indicator. In the absence of chromic ion, the results were high by 1.3 parts per 1000. In the presence of 0.16 *M* chromic ion, the error increased to 3.3 parts per 1000. When the indicator correction was applied this error was diminished to 2.0 parts per 1000.

DESIGN OF TITRIMETER

The instrument used, shown in section in Figure 1, is a light-tight box, *A*, with black interior walls, provided with a stirrer, *C*, a buret, *B*, and an opening, *F*, for observing the end point. The opening is so constructed that no light can pass into the box when the observer's eyes are at the opening. The stirrer may be either motor-driven or hand-operated.

SILOXENE INDICATOR

The structure, preparation, and behavior of the siloxene indicator employed have been described (*4*). The chemistry

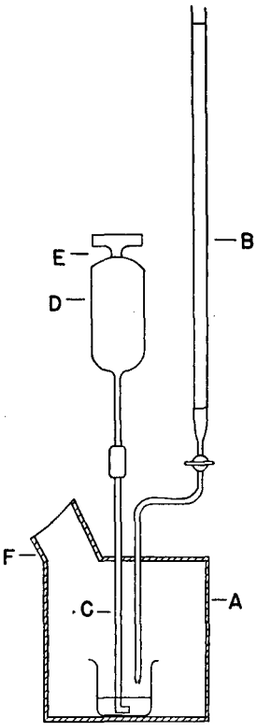

Figure 1. Dark-Chamber Titrimeter

Table I. Volume of Ceric Solution Equivalent to 30.00 Ml. of Ferrous Solution at 23° ± 2° C.

Titration	Ceric Solution, Ml.	Deviation from Mean, Ml.	Potential Difference (from Curves), Mv.	Deviation from Mean, Mv.
1	29.88	0.01	753	2
2	29.88	0.01	753	2
3	29.84	0.03	746	5
4	29.84	0.03	753	2
5	29.88	0.01	753	2
6	29.89	0.02	752	1
7	29.88	0.01	748	3
8	29.87	0.00	753	2
Mean	29.87	0.015	751	3

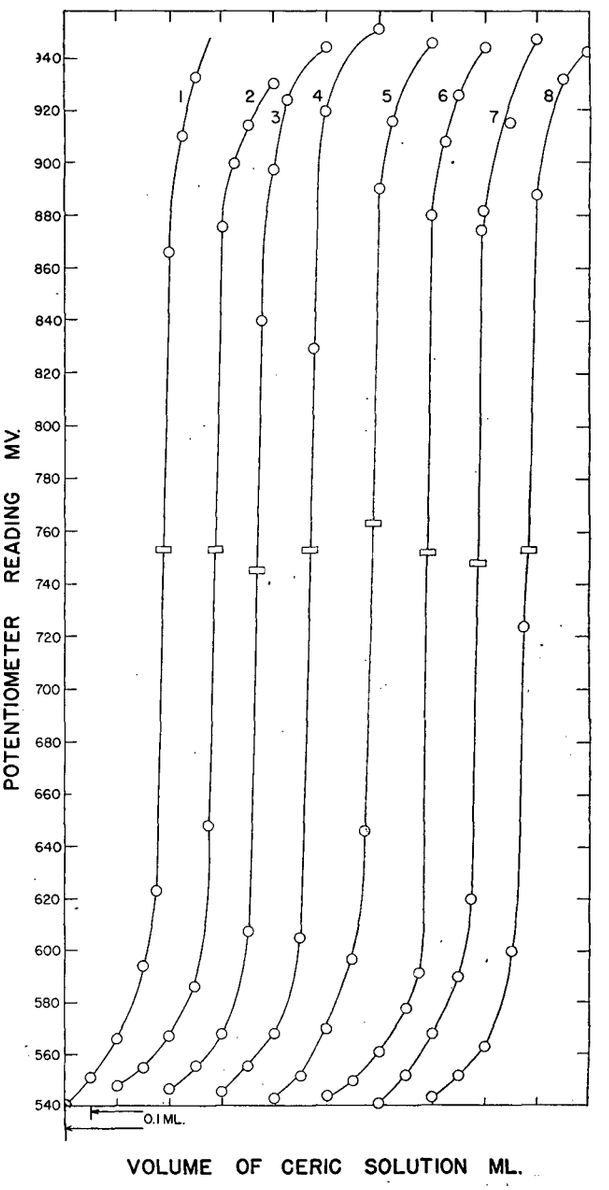

Figure 2. Titration Curves Used in Locating Soitchiometric Point

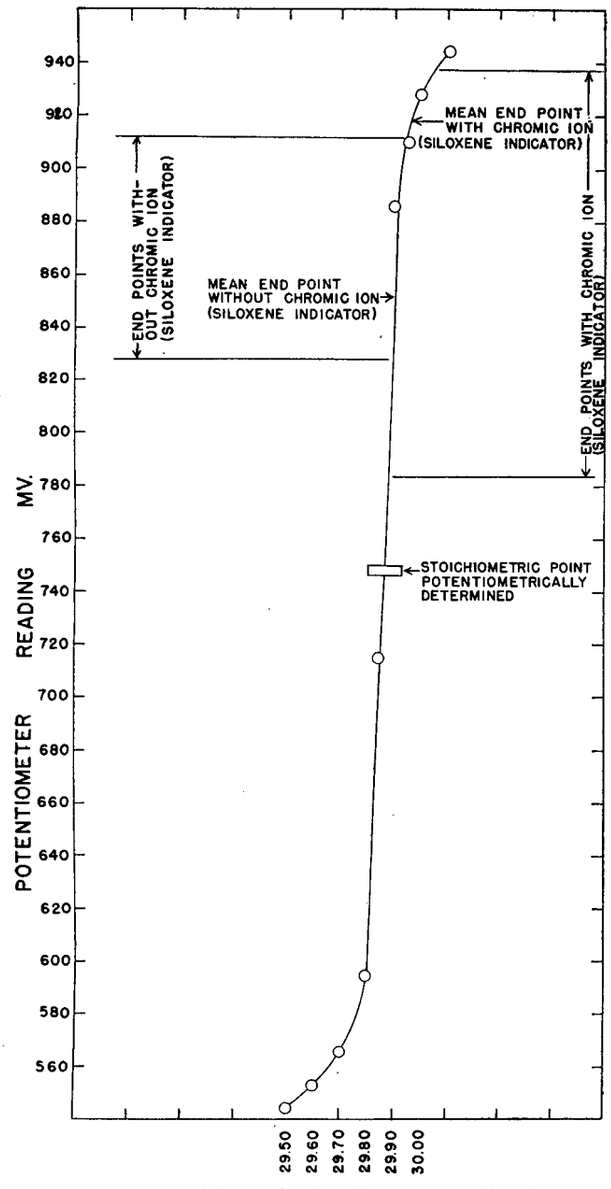

Figure 3. Average Curve Showing Range of End Points with and without Chromic Ion

of siloxene and its derivatives has been discussed by Kautsky (*3*), and brief summaries in English are to be found in some treatises on inorganic chemistry (*1*, *2*). In the titration of ferrous solution with ceric sulfate solution, each drop of ceric sulfate added produced a momentary bright spot. When the stoichiometric point was reached, the sudden increase of oxidation potential brought about reactions within the suspended indicator particles which caused light to come from all parts of the solution and to persist for a considerable time. This was taken as the end point.

EXPERIMENTAL

Approximately 0.1 *M* solutions of ferrous ammonium sulfate and ceric sulfate were prepared. These solutions were compared by titrating eight 30.00-ml. portions of the ferrous solution with the ceric solution, using a gold indicator electrode, a saturated calomel reference electrode, and a Leeds and Northrup 7660-A vacuum tube potentiometer at 23° ± 2° C. Because the curve for the reaction is symmetrical with respect to the stoichiometric point, the midpoint of the nearly vertical portion was taken as the end point.

The eight curves are shown in Figure 2 and the mean curve in Figure 3. As shown in Table I, the curves of Figure 2 yield an average value of 29.87 ml. for the volume of ceric sulfate required to reach the end point, with an average deviation of ±0.015 ml., which corresponds to a precision of 0.50 part per 1000.

In order to test the dark-chamber Titrimeter and compare its performance with that of the dark room (*4*), five 30.00-ml. portions of the ferrous solution were titrated in the dark chamber with the ceric solution, using 100 mg. of siloxene indicator. The results are shown in Table II.

In order to test the effectiveness of the indicator in the presence of a highly colored component, five 30.00-ml. portions of the ferrous solution were titrated in the dark chamber with ceric solution, using siloxene indicator. Solutions titrated were 0.16 *M* with respect to chromic ion and had sufficient depth of color to make the use of any of the ordinary redox indicators utterly impossible. The results are set forth in Table II.

When chromic ion is absent, the average deviation of a single observation of 0.02 ml. corresponds to a precision of 0.67 part per 1000. The indicator error—namely, 29.91 minus 29.87 or 0.04 ml. —is the same as the value previously reported (*4*) when a dark room instead of a dark-chamber Titrimeter was used. In the present work this error corresponds to an accuracy of 1.3 parts per 1000.

When chromic ion is present, the average deviation of a single observation of 0.05 ml. corresponds to a precision of 1.6 parts per 1000. The indicator error—namely, 29.97 minus 29.87 or 0.10 ml.—corresponds to an error of 3.3 parts per 1000. The accuracy of this titration could be improved by using siloxene in standardizing the ceric solution or by applying the indicator correction. If the indicator correction were applied, the accuracy would become 2.0 parts per 1000.

The difference of potential at the end point when the ceric sulfate was standardized and at the end point when siloxene indicator was used in the absence of chromic ion—namely, 848 minus 751 or 97 mv.—agrees fairly well with the value of 87 mv. previously reported (*4*). When the solution titrated was 0.16 *M* with respect to chromic ion, the difference was 861 minus 751 or 110 mv.

Table II. Volume of Ceric Solution Equivalent to 30.00 Ml. of Ferrous Solution and Corresponding Potentiometer Readings Using Siloxene Indicator at 23° ± 2° C.

	In Absence of Chromic Ion				In Presence of 0.16 *M* Chromic Ion			
	Ceric solution, ml.	Dev. from mean, ml.	Potentiometer reading, mv.	Dev. from mean, mv.	Ceric solution, ml.	Dev. from mean, ml.	Potentiometer reading, mv.	Dev. from mean, mv.
	29.95	0.04	831	17	29.88	0.11	887	11
	29.90	0.01	848	0	29.90	0.07	810	51
	29.90	0.01	884	36	30.01	0.04	864	3
	29.92	0.01	827	21	30.00	0.03	800	61
	29.90	0.01	852	4	30.05	0.02	942	81
Mean	29.91	0.02	848	18	29.97	0.05	861	41

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Spectrophotometric Analysis of Amithiozone Preparations

GABOR B. LEVY AND DAVID FERGUS

Schenley Laboratories, Inc., Lawrenceburg, Ind.

AMITHIOZONE (proposed generic name for *p*-acetylaminobenzaldehyde thiosemicarbazone) was introduced as an antitubercular agent by Domagk, Behnisch, Mietzsch, and Schmidt in 1946 (*1*). It is gradually finding wider use (as Tibione, Conteben, and under other trade names) and therefore a rapid method for its determination is desirable. Wollenberg has developed several techniques of analysis (*3*). By one, the compound is oxidized and determined volumetrically. The other type of determination is based on the formation of colored compounds from breakdown products which can be determined colorimetrically. A somewhat greater degree of specificity can be achieved by determining the unchanged molecule. Therefore, the spectrophotometric method for the determination of thiosemicarbazones, described by Spinks (*2*), was investigated. It was found that amithiozone has a marked absorption in methanol at 3280 A. and, at that wave length, Beer's law is valid.

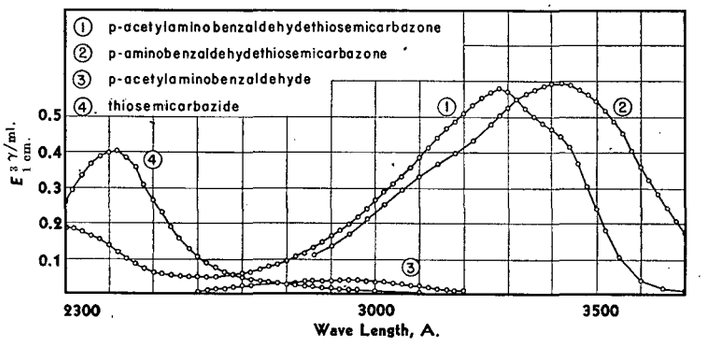

Figure 1. Absorption Spectra

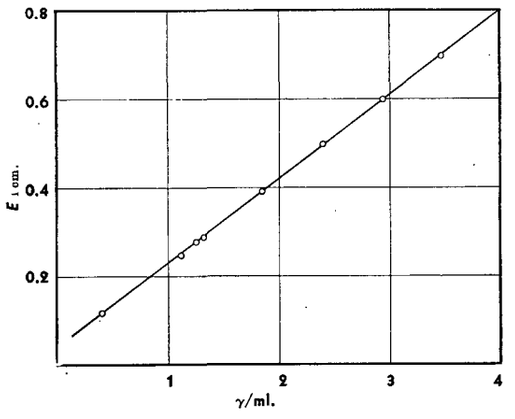

Figure 2. Calibration Curve

On this basis a rapid method for the determination of amithiozone in powders and tablets was developed. The accuracy of this technique is enhanced by the fact that some breakdown or hydrolytic products of the compound show no ultraviolet absorption at 3280 A. This is represented in Figure 1, where the ultraviolet absorption of *p*-acetylaminobenzaldehyde thiosemicarbazone (Tibione brand of amithiozone) is shown together with that of *p*-acetylaminobenzaldehyde and thiosemicarbazide. In the same table (curve 2) is also included a portion of the ultraviolet absorption curve of *p*-aminobenzaldehyde thiosemicarbazone, which is a possible hydrolytic product. However, it has not been found by the authors, by chemical tests, to be present in commercial preparations. Therefore, in practice, determination of the amithiozone content quantitatively at 3280 A. is recommended using the calibration curve shown in Figure 2. If it is desirable to confirm the identity and ascertain the absence of impurities—e.g., that of the *p*-amino compound—the absorption at a number of additional spectral points may be determined simultaneously. As an example, in Table I the relative absorption at 14 wave lengths is shown. It was found that in the range of 2300 A. the absorption is markedly sensitive to the presence of impurities, small amounts increasing the absorption disproportionately. The small secondary absorption maximum of amithiozone at 3400 A. is not due to impurity (the *p*-amino compound), because this maximum is shifted to 3500 A. in benzene while that of the *p*-amino compound is at 3420 A.

To analyze the commercial products the procedure described herewith is used.

Table I. Relative Absorption of Amithiozone

(*p*-Acetylaminobenzylaldehyde thiosemicarbazone)

Wave Length, A.	Relative Spectral Absorption, $\frac{(EX)}{(E\ 3280\ A.)}$
2300	0.326
2600	0.081
2800	0.158
2900	0.276
3000	0.451
3100	0.657
3200	0.872
3260	0.986
3280	1
3300	0.981
3400	0.797
3500	0.411
3600	0.062
3700	0.01

Table II. Replicate Analyses

Amithiozone Powder

	Dilution, mg./l.	*E*	Purity, %
1	70.4/25	0.527	99.01
2	63.7/20	0.599	99.51
3	69.1/25	0.521	99.74
4	65.9/20	0.621	99.72
5	75.4/25	0.568	99.65
6	57.1/20	0.535	99.15
7	69.0/25	0.515	98.73
8	57.1/20	0.535	99.15
9	63.8/20	0.594	98.52
10	63.6/20	0.599	99.66
		$\bar{X}$ =	99.28%
		σ =	0.42%
		v =	0.42%

Tibione Brand Amithiozone Tablets

	Tablet weight, g.	*E*[a]	Tibione Mg./tablet	Tibione Mg./100, mg.
1	0.0926	0.426	45.0	48.6
2	0.1007	0.461	48.8	48.5
3	0.0970	0.453	48.0	49.5
4	0.0936	0.433	45.8	48.9
5	0.1069	0.500	52.9	49.5
6	0.0965	0.444	47.0	48.7
7	0.1011	0.461	48.8	48.3
8	0.1008	0.468	49.6	49.2
9	0.0991	0.459	48.6	49.0
10	0.0987	0.449	47.6	48.2
			$\bar{X}$ =	48.8 mg.
			σ =	0.46 mg.
			v =	0.94%

[a] Dilution in all samples to 20 liters.

Macerate a tablet containing amithiozone with pure synthetic methanol in a mortar with the pestle. Repeat three times, and each time decant through a filter into a volumetric flask. In the case of essentially pure powder dissolve in a corresponding volume of methanol, omitting maceration. Use enough methanol to have an approximate concentration of not more than 0.5 mg. per ml. Make a secondary dilution in methanol, so that a final dilution of 3 ± 1 micrograms per ml. results. Using the same methanol as a blank, determine the extinction at 20° C. at 3280 A., using 1-cm. cells and a Beckman DU or similar instrument.

In Table II two sets of ten replicate results are listed: analyses of an essentially pure preparation and of a single commercial lot of tablets. The latter results are corrected for tablet weight. Similar analyses were performed on experimental and commercial products of a number of manufacturers, of both foreign and domestic manufacture. No evidence of interference was found. The precision is comparable to other spectrophotometric methods. The extraction step, required for tablet analysis, reduces precision somewhat. The technique is recommended for the routine analysis of amithiozone products.

ACKNOWLEDGMENT

The authors are indebted to Bruno Puetzer for suggesting this problem and for discussions, for Kurt Ladenburg for advice and encouragement, and to Schenley Laboratories, Inc., for permission to publish this note.

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Modification of Sanchez Color Test for Nicotine

Application to Nicotine, Nornicotine, and Anabasine

LOUIS FEINSTEIN AND EDWARD T. McCABE

Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md.

IN a search of the literature on tobacco alkaloids, it was noted that Sanchez (*3*) gave a color test for nicotine using vanillin and strong hydrochloric acid, the color varying from a rose red to a deep cherry red, according to the nicotine concentration. Sanchez's test is not directly a test for nicotine and this note presents the authors' experience with this test when it is applied only to nicotine, nornicotine, and anabasine (*4*). These three alkaloids are perhaps not the only ones which can be made to give colors in this test, but Sanchez's test, when correctly applied, can be used to provide additional information in a study of these three alkaloids. Anabasine is an isomer of nicotine, both being closely related physiologically. Both alkaloids are liquids, forming picrates of melting points 212–213° and 223–224° C., respectively, for anabasine and nicotine. Sirupy 85% orthophosphoric acid has been used because of convenience in place of strong hydrochloric acid in the Sanchez test.

In experiments with vanillin–phosphoric acid, carefully purified samples of nicotine and nornicotine failed to give the Sanchez test, but anabasine (extracted out of *Nicotiana glauca*) gave a faint brown-violet color. Further experiments showed that the following compounds (all having a six-member ring containing nitrogen) failed to give a red color with vanillin and phosphoric acid: anabasine, piperidine, γ-dipyridine, 3-aminopyridine, benzoyl piperidine, 3-bromopyridine, and acridine. Any change in color appearing is toward a yellow or brown-violet. Pyrrol, a compound with a simple five-member ring containing nitrogen, gives an immediate red color in dilute concentration, while carbazole, where the five-member ring containing nitrogen is bound at each carbon as part of benzene rings, gives the red-color reaction slowly (after being stirred 20 to 70 minutes). Pyrrolidine gives no color change with vanillin and phosphoric acid. Myosmine crystals change to red when vanillin–phosphoric acid solution is dropped upon them. Nicotyrine gives an immediate deep red color with vanillin and phosphoric acid solution.

The authors then dehydrogenated nicotine and nornicotine, using platinum-black prepared by the method of Linstead and Thomas (*1*), and found that the dehydrogenated samples gave red color solutions with vanillin and phosphoric acid. Anabasine does not give this red color before or after a similar treatment.

The procedure for dehydrogenating and reacting with vanillin and phosphoric acid is as follows:

A small quantity of the alkaloid is mixed with a quantity of the platinum-black and heated in a vessel to 170° C. for 1.5 hours, taking care to prevent the alkaloid from evaporating off. After the sample is cooled, 5 ml. of distilled water are added and the whole is filtered. To 3 ml. of the filtrate are added 12 ml. of the vanillin–phosphoric acid solution (0.5 gram of vanillin in 100 ml. of 85% orthophosphoric acid at room temperature) and the color change is noted.

A somewhat different test is that using Meltzer's reagent, which is made up of carbon disulfide, ethyl alcohol, and dilute copper sulfate solution. Razvadovskiĭ (*2*) used this reagent to distinguish nicotine from anabasine. Nicotine gives a water-white solution or slight turbidity with Meltzer's reagent. Anabasine gives a brown-black solution. This test will also show the presence of nornicotine, which reacts like anabasine, giving a brown-black solution. Meltzer's reagent can be used to show the absence of nornicotine and anabasine in nicotine. The presence of nornicotine, anabasine, or both in nicotine will cause a blackening of the reagent solution.

To use the reagent, 5 ml. of carbon disulfide are mixed with 95 ml. of ethyl alcohol and 20 ml. of this mixture are added to 0.05 ml. of alkaloid. After a thorough mixing, 2 drops of 20% copper sulfate solution are added and the mixture is shaken.

ACKNOWLEDGMENT

The authors wish to thank Abner Eisner, Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Philadelphia, Pa., for samples of pyrrolidine and myosmine.

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RECEIVED June 22, 1950. Presented before the Division of Biological Chemistry at the 117th Meeting of the AMERICAN CHEMICAL SOCIETY, Philadelphia, Pa. Report of a study made under the Research and Marketing Act of 1946.

Reference Standard for Mass Spectrometric Analysis of Nitrogen

SIDNEY SOLOWAY

Division of Nutrition and Physiology,
Public Health Research Institute of the City of New York, Inc., New York, N. Y.

IN THE course of routine mass spectrometric analyses of nitrogen variously enriched with N^{15}, it was necessary to have available at all times a sample of standard nitrogen both for reference purposes and for sweeping after the analysis of highly enriched samples. Desirable features of such a standard would be: (1) constancy of isotopic composition, (2) ease of preparation, and (3) freedom from oxygen, because it has been found that oxygen disturbs the emission characteristics of tungsten filaments.

Table I. Comparison of Composition of Samples of Nitrogen Prepared by Various Means

Source of N_2	No. of Samples	N^{15}, Atom %	Oxygen, Vol. %	Argon, Vol. %
Air + Fieser's soln.	4	0.382 ± 0.001	<0.01	0.8
Commercial N_2	5	0.382 ± 0.001	0.3–0.5	0.4
$(NH_4)_2SO_4$ + NaOBr	6	0.382 ± 0.001	0.1–0.3	0

Samples of nitrogen meeting these requirements have been prepared by the exposure of room air to Fieser's solution (*1*), which readily absorbs oxygen.

Fieser's solution is made by adding 2 grams of sodium anthraquinone β-sulfonate and 15 grams of sodium dithionite to 20 grams of potassium hydroxide in 100 ml. of water. Five milliliters of this solution are placed in a 15-ml. bulb equipped with a stopcock, and the stopcock is then closed. After 2 hours, the flask is chilled in a dry ice bath and the gas is admitted into the mass spectrometer. One such exposure to air has been found sufficient for four or five nitrogen analyses, and the number of times a given charge of Fieser's solution could be exposed to air would depend on the care of its preparation, normally four or five times before a mass 32 peak could be detected.

The atom per cent N^{15} was calculated from the ratio of masses 28/29 determined directly by the mass spectrometer, using a system of magnetic scanning. The analytical values were reproducible with a probable error of 0.001 atom %, as shown in Table I. The difference of approximately 5% between the natural abundance of N^{15} reported herein and that recently reported by Nier (*2*) may be attributed to differences in the scanning technique and in the arrangement of the slits in the ion gun.

From Table I it can be seen that normal nitrogen samples prepared by three methods contain, within the precision of the author's instrument, identical abundances of N^{15}. Samples prepared by the method described herein contain no detectable oxygen, which is a constant contaminant in samples of tank nitrogen as well as in samples prepared from ammonium sulfate (*3*). The method has proved much more rapid and convenient in this laboratory than either of the other two methods, both for sweeping and as a standard. In addition, the simple removal of oxygen from normal air ensures the absence of any fractionation of the nitrogen isotopes. Whereas no evidence of such fractionation in either tank nitrogen or ammonium sulfate has been encountered thus far, the occurrence of such fractionation in the preparation of these materials may become detectable as the precision of mass spectrometers increases.

ACKNOWLEDGMENT

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Electrolytic Determination of Antimony

GEORGE NORWITZ

Laboratory of George Norwitz, 3353 Ridge Ave., Philadelphia 32, Pa.

THE methods proposed for the electrolytic determination of antimony at constant current have little to recommend them. Deposition from acidic solutions has not been successful because of the difficulty of obtaining adherent deposits and complete deposition (*6*, *7*). Deposition from sulfide solutions gives results that are 1 to 1.5% too high, owing to the occlusion of oxygen and sulfur compounds (*1*, *3*, *6*, *7*).

After an investigation of the deposition of antimony from many different electrolytes, an accurate electrolytic method for determining this element was developed by the author, in which the antimony is electrolyzed from a sulfuric-hydrochloric acid solution containing hydroxylamine hydrochloride. The latter reagent has been used for the electrolytic determination of antimony at controlled potential from a hydrochloric acid solution, but the methods proposed (*2*, *5*) require careful control of the temperature as well as potential. In the method of the author neither the temperature nor the potential is critical. For the successful deposition of antimony at constant current the antimony must be present in the antimonic state. Hydrazine sulfate was found to be ineffective as an addition agent.

Table I. Determination of Antimony

Antimony Present *Gram*	Antimony Found *Gram*	Antimony Present *Gram*	Antimony Found *Gram*
0.1000	0.1001	0.3000	0.2996
0.1000	0.1002	0.3000	0.3005
0.1000	0.0995	0.3000	0.3001
0.2000	0.2002	0.4000	0.4008
0.2000	0.1996	0.4000	0.4010
0.2000	0.1997	0.4000	0.4007

EXPERIMENTAL

Various amounts of pure metallic antimony were dissolved in 15 ml. of sulfuric acid (97%) by heating on the hot plate, and the solutions were cooled and diluted to 125 ml. with water. Ten milliliters of hydrochloric acid (38%) and 10 ml. of hydrogen peroxide (3%) were added and the solutions were boiled for 10 minutes to destroy the hydrogen peroxide. The solutions were

diluted to 190 ml. and 5 grams of hydroxylamine hydrochloride were added. The solutions were then electrolyzed for 1 hour, using platinum gauze cathodes and platinum spiral anodes. Two amperes (per sq. dm.) were used during the first 15 minutes and 1 ampere (per sq. dm.) for the last 45 minutes. The electrolytes were stirred by air circulation. After the electrolysis the cathodes were immersed in water and alcohol, dried at 105° C. for 3 minutes, cooled, and weighed. The current used by the author had an impressed voltage of 9 volts. The voltage across the electrodes was 2.8 volts. The results obtained for antimony are shown in Table I.

DISCUSSION

When metallic antimony is dissolved in sulfuric acid, the antimony is in the antimonious state and must be oxidized with hydrogen peroxide. There is little danger of losing antimony by volatilization when the solution is boiled to destroy the hydrogen peroxide (*4*).

The amount of sulfuric acid and hydrochloric acid used in the method is not critical. Good results were obtained using 10 to 20 ml. of sulfuric acid and 5 to 20 ml. of hydrochloric acid. The exact temperature used during the electrolysis is not important. Equally good results were obtained using temperatures varying from room temperature to 80° C.

Tests made with hydrogen sulfide after the electrolyses showed that no detectable amounts of antimony were left undeposited. It was not feasible to test the deposits for possible occlusion of chlorine, hydrogen, and oxygen. However, the fact that excellent results were obtained indicates that the deposits were very pure.

More than 0.4 gram of antimony should not be determined by the method; otherwise high results will be obtained. For instance, the result obtained by the author in electrolyzing 0.5000 gram of antimony was 0.5024 gram. In the sulfide method 0.2 gram of antimony is the maximum amount that can be handled (*6*).

Copper, cadmium, tin, arsenic, lead, bismuth, and silver interfere with the method, and when they are present, preliminary separations are necessary.

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RECEIVED April 21, 1950.

Versatile Paper Partition Chromatographic Apparatus

ARNOLD J. SINGER AND LEONARD KENNER
Amm-i-dent, Inc., Jersey City 6, N. J.

THE past few years have seen the increased use of paper partition chromatography as both a qualitative and a quantitative analytical micromethod. Coincident with increasing use of this method has been improvement of the apparatus in which the chromatogram is developed. Methods previously introduced have embodied the principle of capillary descent (*1, 2, 5, 8, 10*) and capillary ascent (*4, 7, 9, 11*). Numerous devices for the phase of solvent flow separation have been described, but these provide inadequate space (*6, 7*), have the paper strips inadequately supported (*3, 9, 11*), or have both disadvantages (*4, 7*).

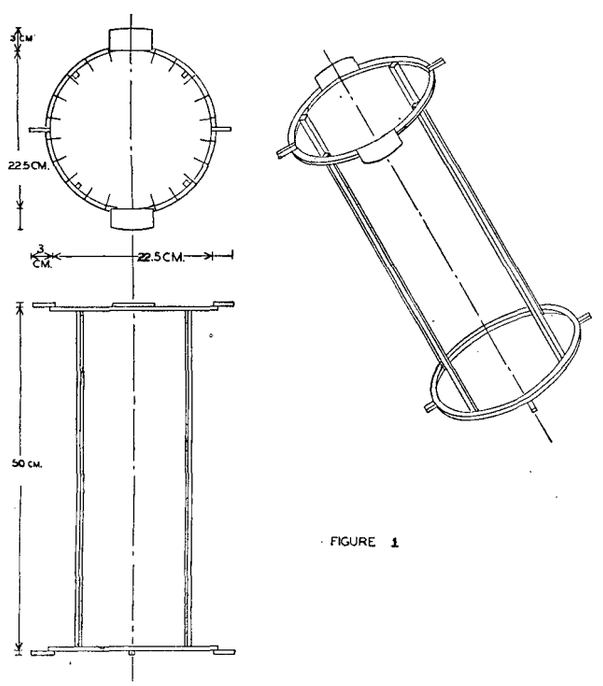

FIGURE 1

The authors have constructed a frame for rigid support of the paper at both ends during the phase of solvent separation by capillary ascent. The frame has the following advantages in the use of paper partition chromatography as an analytical or separatory procedure: Up to 50 one-dimensional chromatographs can be developed at one time; contamination of the strips by contact with each other or the walls of the apparatus is prevented; there is sufficient vapor space in the apparatus to reduce the effect of slight fluctuations in room temperature on the rate of flow of the developing liquid; the depth of the wick end of the paper in the liquid remains constant; the sample is at a constant distance on the paper from the solvent; and the paper is supported tightly and vertically, which eliminates variations in rate of flow due to slope of the paper strips.

The frame is constructed of 4 × 4 mm. strips of cold-rolled steel. Two strips 73 cm. long were bent into circles having an internal diameter of 22.5 cm. and the ends were brazed together. These circles were then brazed to the ends of four strips 50 cm. long at 90° intervals around the circles. Two 3-cm. lengths of 4-mm. rod were brazed horizontally on the top ring at 180° and two flat "ears" 3 cm. wide, 6.5 cm. long, and 2 mm. thick were brazed on at 90°. Four 3-cm. lengths of rod were brazed horizontally at 90° intervals on the bottom ring. The rods were used as stabilizers to orient the frame in the tank; the ears were used as handles and hangers. Friction tape was wound around both rings and pierced by 2-inch (5-cm.) steel pins pointing toward the center of the frame. The use of tape is suggested for greater flexibility of the apparatus. Similar frames of smaller diameter can be made to telescope within the outer frame. These can be used for additional strips and the frames can be removed selectively at different time intervals.

The developing tank is a borosilicate glass jar, with handles, obtained from A. H. Thomas Co., Philadelphia, Pa. (Catalog No. 6289-A, outside diameter 30.3 cm., inside diameter 29.0 cm., height 60 cm.). The handles are 2.5 cm. from the top of the jar and are formed by indentations of the wall of the jar. These serve as supports for the frame, which is suspended on them by the ears, so that no portion of the frame touches the developing liquid. The top is an ordinary piece of plate glass 12.625 inches square, which is sealed to the jar with a heavy lubricant to give an air-tight seal.

For the chromatogram, paper strips are marked at 4.5 and 8.5 cm. from the end to be immersed in the developing liquid. The sample to be chromatographed is applied at the 8.5-cm. mark and allowed to dry. The frame is suspended from ring stands by its handles outside the tank and each strip is pierced first at the lower or 4.5-cm. mark, stretched taut, and then pierced by the top pin. With all the strips in position, the frame assembly is lowered into the tank until supported by the ears. Care must be taken to prevent contamination of the strips by the tape while being mounted. The glass lid is set in position. When the solvent front has advanced sufficiently, the strips may be pulled off the pins selectively, or the entire frame may be removed from the tank and the strips dried, sprayed, and developed while mounted.

In practice, this apparatus has greatly facilitated chromatographic procedures. It is durable and permits the handling of large numbers of samples during the solvent separation phase of paper chromatography.

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Improved 5-Mg. Rider for Ainsworth Microchemical Balances

LAWRENCE E. BROWN

Southern Regional Research Laboratory, New Orleans, La.

IRREGULARITIES in the seating of the 5-mg. wire riders, customarily furnished with older Ainsworth microchemical balances Types FD and FDI, are an important source of error. The magnitude of the seating error can be reduced by using a 0.5 mg. rider, but a sacrifice of convenience is entailed by the lower capacity of the beam. A 5-mg. rider with a low seating error has been constructed of aluminum foil The essential feature of this rider is that it has a thin, straight bearing edge and can be brought to rest in the bottom of the notch of the balance beam with ease and without undue manipulation of the rider carrier.

The rider is made of pure aluminum foil, 0.0035 to 0.004 inch thick. After a 2-inch (5-cm.) square of the foil is covered on both sides with cellulose tape, it is placed on a smooth hard surface and the eye of the rider is cut with a small sharp cork borer (No. 1) by a single light tap. The foil is then flattened between two pieces of plate glass and the irregularities in the circumference of the eye are removed with a 3-mm. conical grinding wheel.

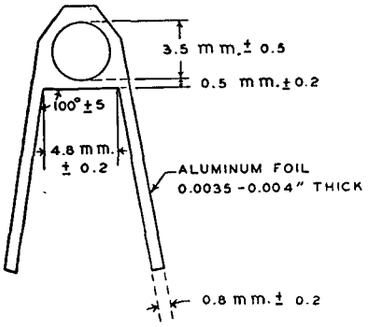


Figure 1. Foil Rider

The coated foil is then placed on a hard smooth surface, such as a piece of plate glass. The cutting edge of a rib-backed razor blade is reduced to a length of 4.8 ± 0.2 mm. and used to cut the cross bar or bearing edge of the rider. The cutting edge of the blade is placed symmetrically about 0.5 mm. from the eye and the cut is made with a single tap on the back of the blade (Figure 1). Similarly, sharp blades are used to cut the insides and outsides of the legs, the outside shoulders, and the outside of the eye arc. The most critical points are the inside corners. The legs should be about 1-mm. wide and the angle with the bearing edge about 100°.

The cellulose tape is removed by soaking the formed rider in acetone. The legs are trimmed in length until the rider weighs about 5.1 mg. The final adjustment to exactly 5 mg. is made by dissolving some of the aluminum by immersion in 0.1 *N* sodium hydroxide, which attacks microscopic burrs and imperfections preferentially. After final cleaning and drying, the rider is flattened by pressing between pieces of plate glass. It is then ready for use.

Table I. Standard Deviations of Observations

Rider	Observed for Pan Arrestment and Rider, $x + y^a$ γ	Calculated for Rider Alone, y^b γ
5-mg. wire rider	1.9	1.7
5-mg. foil rider	1.3	1.0
0.5-mg. wire rider	1.2	0.9

[a] $(x + y) = s$ observed for pan arrestment and rider. $(x) = s$ observed for pan arrestment alone = 0.8 γ. $n = 30$ observations in all cases.
[b] $y = s$ calculated for rider seating alone as square root of differences of squares of $(x + y)$ and (x).

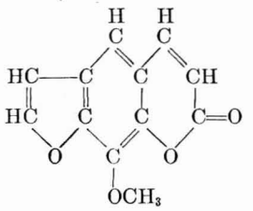
The performance of the foil rider compared with that of the riders supplied by the manufacturer, using an Ainsworth Type FDI microchemical balance, is indicated by the data given in Table I. The reading error on the optical lever scale was assumed to be negligible. The standard deviation reported for the 5-mg. wire rider is for the best series of observations attained by the author after considerable experimentation in rider seating. Unless strict care is used in seating, a much larger standard deviation occurs. The foil rider is adequately seated by a vertical drop of it from the carrier. Further manipulation, such as touching or rocking the seated rider with the tip of the carrier, results in lower precision. The difference found between the standard deviations of the 5-mg. foil and wire riders is statistically significant at the 95% level. The other standard deviations observed are considered typical and indicate that the 5-mg. foil rider has a seating error comparable to that of a conventional 0.5-mg. rider.

RECEIVED July 6, 1950.

CRYSTALLOGRAPHIC DATA

41. Xanthotoxin I

Contributed by JOHN KRC, Armour Research Foundation, Illinois Institute of Technology, Chicago 16, Ill.



Structural Formula for Xanthotoxin

Good crystals of xanthotoxin I can be obtained by crystallization from ethyl alcohol, although benzyl alcohol is the best solvent for recrystallization on a microscope slide (Figure 1,*a*). Characteristic crystals from the melt are shown in Figure 1,*b*. Figure 2 is an orthographic projection of a typical crystal of xanthotoxin I. Xanthotoxin is almost insoluble in cold water and only 0.1% soluble in boiling water. It is only slightly soluble in petroleum ether, freely soluble in chloroform, and less soluble in benzene, alcohol, and ether.

Xanthotoxin II can be obtained fairly readily on a microscope slide by thoroughly melting a pure sample, followed by chilling on a cold surface. There is no evidence that modification II can be formed from solution and it is doubtful that such an unstable form could be crystallized from solution.

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic.

Form and Habit. Rods and needles elongated along *c*, showing forms {100}, {001}, {110}, and {801}.

Axial Ratio. $a:b:c = 0.818:1:0.307$.

Interfacial Angles (Polar). $110 \wedge \bar{1}10 = 101\frac{1}{2}°$; $110 \wedge 100 = 39\frac{1}{4}°$.

$801 \wedge \bar{8}01 = 143°$.

Cleavage. Excellent parallel to 010 and 100.

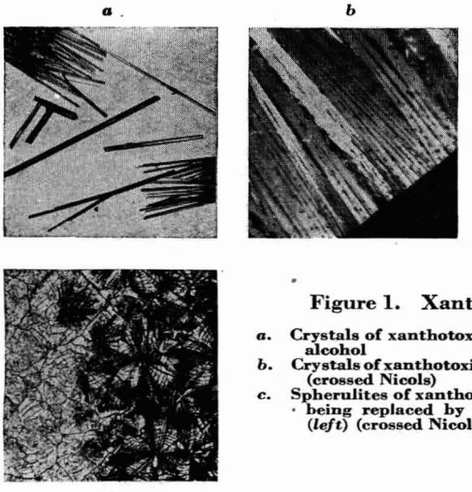


Figure 1. Xanthotoxin

a. **Crystals of xanthotoxin I from ethyl alcohol**
b. **Crystals of xanthotoxin I from fusion (crossed Nicols)**
c. **Spherulites of xanthotoxin II (*right*) being replaced by xanthotoxin I (*left*) (crossed Nicols)**

X-RAY DIFFRACTION DATA

Cell Dimensions. $a = 12.95$ A.; $b = 15.83$ A.; $c = 4.86$ A.

Formula Weights per Cell. 4.

Formula Weight. 216.

Density. 1.449 (flotation-centrifugation-density balance); 1.440 (x-ray).

Principal Lines

d	I/I_1	*d*	I/I_1
10.08	0.06	2.58	0.02
7.89	0.10	2.54	0.02
6.60	0.62	2.44	0.04
4.97	0.08	2.38	Very weak
4.62	0.06	2.30	0.02
4.35	0.38	2.24	0.03
4.14	0.06	2.17	0.03
3.93	0.09	2.05	0.06
3.76	0.20	1.954	0.02
3.56	0.57	1.896	0.06
3.43	1.0	1.871	0.03
3.32	Very weak	1.838	0.03
3.22	Very weak	1.781	Very weak
3.10	0.06	1.755	0.06
2.97	0.26	1.715	0.03
2.88	Very weak	1.673	Very weak
2.73	0.05	1.603	Very weak

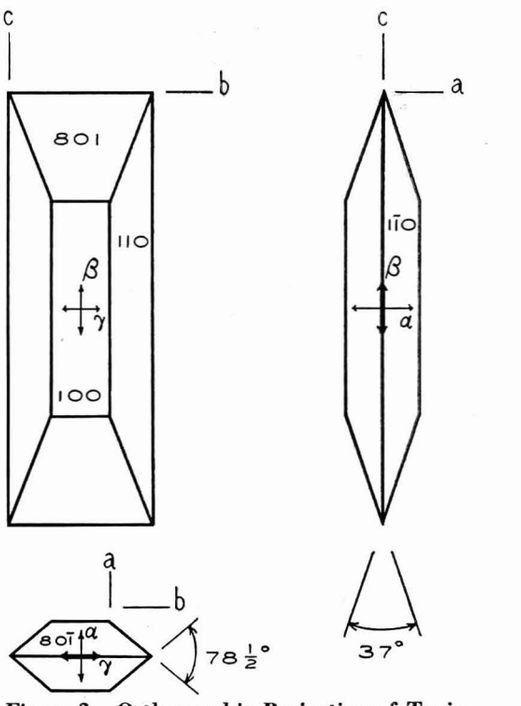


Figure 2. Orthographic Projection of Typical Crystal of Xanthotoxin I from Ethyl Alcohol

OPTICAL PROPERTIES

Refractive Indexes (5893 A.; 25° C.). $\alpha = 1.698 \pm 0.003$. $\beta = 1.734 \pm 0.006$. $\gamma = 1.742 \pm 0.006$.

Optic Axial Angles (5893 A.; 25° C.). $2H = 48\frac{1}{2}°$. $2V = 43°$ (calcd. from β and $2H$); 48° (calcd. from α, β, and γ). $2E$ = ca. 82°.

Dispersion. $r > v$, very strong.

Optic Axial Plane. 001.

Sign of Double Refraction. Negative.

Acute Bisectrix. $a = \alpha$.

Extinction. Parallel and symmetrical.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.724$. R (calcd.) = 57.1. R (obsd.) = 59.2.

FUSION DATA. Xanthotoxin I melts at 148° C. with some sublimation and slight decomposition. When cooled just below its melting point, it crystallizes as highly birefringent rods. If cooled rapidly to room temperature, a lower temperature modification, xanthotoxin II, may be obtained, crystallizing as low birefringent crystals radiating spherulites from a dozen or more centers of nucleation. This modification, II, is very unstable and is replaced on rewarming by the stable and highly birefringent modification, xanthotoxin I. The transformation II → I is extremely slow below 40° C. for the pure compound and increases with temperature until it becomes rapid at 70° C. (and above).

The transformation I → II was not observed. Figure 1,*c*, shows crystals of xanthotoxin I (*left*) replacing spherulites of xanthotoxin II (*right*). A solution phase transformation II → I can be readily observed at room temperature by surrounding the crystals with benzyl alcohol in a preparation including both modifications. This preparation is obtained by heating one side of a preparation of modification II and immediately cooling to slow the transformation to I. In general, modification II transforms entirely to I well below the melting point.

ACKNOWLEDGMENT

The author is indebted to R. L. Felton and the Paul B. Elder Co. for the sample of xanthotoxin used in this investigation and for solubility data.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill

Book Review

Analytische Chemie der Düngemittel. *Siegfried Gericke.* 191 pages. Die Chemische Analyse, Vol. XLIV. Ferdinand Enke, Stuttgart, 1949. Price, paper $5.62, linen $6.19.

Analytical procedures are described in detail for liming materials, phosphate, potassium, and nitrogen fertilizers, fertilizers of organic origin, and mixed goods used in Germany. The treatment of phosphates covers basic slag, superphosphate, Rhenania phosphate, dicalcium phosphate, phosphate rock, bone meal, and new phosphates; that of nitrogen materials covers nitrate, ammonia, and amide nitrogenous products. Descriptions of the various materials, given in appropriate places, include method of production, grade specifications, fineness, and chemical composition shown by typical analyses. The status of fertilizer research in Germany, as of 1948, is discussed, and summarized data on national fertilizer consumption and use are given. The text closes with a discussion of fertilizers as a basis of nutritional improvement, in which are included graphical comparisons of trends in infant mortality, average life span, and cancer death rate with the trend in fertilizer consumption. The author has incorporated in a book of control methods sufficient material of wider interest to afford a brief coverage of German fertilizer production, evaluation, and use.

J. H. CARO

Symposia Committee

The terms of L. T. Hallett and Beverly L. Clarke on the Committee on Annual Symposia have expired, and Louis Gordon, Syracuse University, and James M. Crowe, ANALYTICAL CHEMISTRY, have been appointed in their places. The committee now consists of P. J. Elving, chairman, J. W. Stillman, Edward Wichers, I. M. Kolthoff, Louis Gordon, and J. M. Crowe.

Fourth Symposium on Analytical Chemistry

THE fourth annual Symposium on Modern Methods of Analytical Chemistry was held January 29 to February 1 at the Louisiana State University, Baton Rouge, La. Abstracts of the papers presented are reproduced here.

Oscillographic Polarography. PAUL DELAHAY, Louisiana State University.

Methods of Measurement. Single-sweep methods. Multisweep methods
Randles-Sevcik equation
Causes of error
  Resistance of the circuit
  Capacity current
  Irreversibility
  Other causes of error (multisweep method)
Applications
  Routine determinations
  Analyses in the micromolar concentration range
  Kinetics studies
Use of platinum microelectrodes
Comparison with conventional polarography
Demonstrations

Nonmagnetic Radiofrequency Mass Spectrometer. W. H. BENNETT, University of Arkansas.

A new kind of mass spectrometer has been developed at the National Bureau of Standards in which the masses are separated by velocity rather than magnetic deflections of focused beams. The principles of this new kind of mass spectrometer were presented.

This instrument is very different from previous instruments in several respects. First, it is simpler, more rugged, and much less expensive. Secondly, it is much more sensitive, giving currents at the collector about 10,000 times as large as those of the more familiar instruments. Thirdly, the instrument does not use slits and because of this absence of slits the instrument can be adapted to a great variety of applications where previous instruments could not be used at all.

A number of typical applications where this instrument enjoys unique advantages were mentioned.

Recent work on the development of the instrument has resulted in an improved understanding of the factors determining the resolution and sensitivity. Some recent developments in respect to the ion sources were presented.

The various processes which are responsible for background currents were discussed.

Polarographic Behavior of Organic Compounds. PHILIP J. ELVING, Pennsylvania State College.

Principles of measurement and interpretation
  Experimental arrangement and conditions: apparatus, sample solution
  Nature of record obtained: the polarogram
  Significance of fundamental equations: Ilkovič equation and equation of the wave
  Current-controlling processes
  Deduction from experimental data
Applicability of polarography to organic compounds
  Electroactive functional entities: reducible, oxidizible, and nonrelative groups
  Case history: the carbonyl group
  Effect of substituents in the molecule
Current development in organic polarography
  Study of nature of phenomena involved
    1. Buffers, pH, ionic strength
    2. Reaction and electrode mechanisms at dropping mercury electrode (oscillographic study)
    3. Nature of irreversible reactions
    4. Oxidative reactions
  Use as an investigative tool
    1. Applications dependent primarily on i_d: analysis, reaction rates, etc.
    2. Applications dependent primarily on $E_{0.5}$: structure, redox potentials, isomerism, etc.
    3. Applications dependent on i_d, $E_{0.5}$, n, etc.: equilibria, hydrogen bonding, etc.
Effect of experimental conditions (environment)
  Concentration of reactive species
  Ionic strength and buffer nature
  Nature of solvent

Polarographic reactivity and reaction mechanisms
  Factors in deduction of a reaction mechanism
  Case history: fission of carbon-halogen bonds
Analytical applications with specific examples
  Basic quantitative techniques
  Determination of elements
  Determination of organic functional groups
  Determination of organic compounds
  Analysis of biologically significant substances
  Amperometric titration
  Coulometric titration
  Partition polarography
  Determination by maximum suppression and by catalytic waves
Other applications
  Reaction rates and kinetic studies
  Structure determination
  Hydrogen bonding
  Synthesis control, detection of reaction intermediates, purification processes

Microchemistry. A. A. BENEDETTI-PICHLER, Queens College.

Inorganic Microanalysis

Definition. Microtechnique of chemical analysis on an essentially smaller scale than customary.

Physical properties and chemical equilibria are in first approximation not dependent upon absolute quantity. Limits were briefly discussed.

The sliding scale: centigram, milligram, microgram techniques. The units were defined.

Practical applications. Research, industrial applications, material testing.

Discussion of microtechniques on centigram and milligram scales. Work on microscope slide, measuring reagents, heating and cooling, separation of phases, weighing, titration.

The advantages and limitations of the use of microtechnique were indicated.

Working with Microgram Quantities

The general technique of working in capillary cones under the microscope was explained with the use of slides. Examples from the fields of inorganic and organic microanalysis were mentioned.

Demonstration of the titrations of microgram quantities under the microscope with the use of a motion picture film.

Brief references to the possibilities for gravimetric and colorimetric determinations on this very small scale.

Microscopy in the Dye Industry. GEORGE L. ROYER, Calco Chemical Division, American Cyanamid Co.

Microscopy has been used for many years by the chemist for the identification of textile fibers and for the examination of defects in fabrics. The advent of color photography has brought a medium to microscopy which can be used to illustrate many microscopical phenomena which in black and white would be meaningless and uninteresting. The dyeing operation for the application of color to textiles is an art to which microscopy has been applied to aid in the advancement of the science of dyeing. In this presentation two slides were projected on separate screens at one time, so that comparisons could be seen readily.

First, the use of chemical petrography was illustrated by photomicrographs of various crystals of intermediates and dyes which result from microscopical tests used for positive identification. Polarized light, refractive index, and other optical properties were shown. The size, shape, and color properties of dyes affect their preparation and use, and here again the microscope is a useful tool to determine these properties.

Dyed common textile fibers were shown in cross section to show the value of the technique for the identification of textile fibers and for the study of fiber structures. This was followed by physical chemical studies on the application of dyes to wool, cotton, and rayon, in which the microscope has played an important part in obtaining a better understanding of the mechanism of dyeing. These studies have been of value in giving a scientific approach to dyeing, an art that has been known for ages. Some ancient fabrics illustrate how dyeing was carried out hundreds of years ago. In conclusion, some photographs were shown on the techniques and apparatus used in these studies and in taking the color photomicrographs.

Mass Spectrometry. R. BOWLING BARNES, American Optical Co.

Infrared Spectrometry

Infrared spectroscopy has passed through its first phase, in which a great deal of fundamental work was done. It is now well along into the second phase—the transition from its use under ideal research conditions to its widespread application under more practical conditions. Whereas it was for a long time thought of only as a narrow branch of spectroscopy, it is now accepted widely as a laboratory tool.

The past few years have seen a tremendous increase in the use of infrared in industry for the solution of chemical problems. One use that has not been so widely publicized as others is its application to the study of molecular structure. Some work has been done in this area but the surface has hardly been scratched.

The necessary basis for successful application of infrared spectroscopy is a knowledge of the absorption spectra of a great many pure compounds. From these absorption spectra, empirical correlations can be made which permit interpretation of the structure of unknown compounds, verification of molecular structures as derived by chemical means, quick accurate analysis of mixtures, determination of impurities, measurement of reaction rates, etc. It is an obvious step to broaden the existing concepts and to summarize spectral correlations with a view to establishing a field of infrared spectroscopy which may be complementary to conventional physical and organic analyses.

The theoretical basis of infrared spectroscopy was presented briefly, along with a discussion of instrumentation and experimental techniques.

A wide variety of applications were then presented. These include discussions of the use of infrared for the determination of spectral identity and qualitative purity; and the application of infrared to qualitative functional group analysis as applied to the establishment of molecular structure.

Recent Trends in Infrared

In the January issue of ANALYTICAL CHEMISTRY, R. C. Gore presented a review of the infrared papers published during 1950. This review contained 354 references. Some of the newer and more important instruments and techniques covered in the above review were described, together with their applications to practical problems in analytical chemistry.

Electron Microscopy. ROBERT FISCHER, University of Indiana.

Principles and Instruments

Requirements to yield a faithful image
  Adequate resolution
    1. Limiting factors
    2. Significance of resolution
  Adequate depth of field
  Adequate image contrast
    1. Origin of contrast
    2. Aids to contrast
Characteristics of a suitable specimen
  Thin enough to transmit an appreciable number of electrons
  Stable in evacuated chamber
  Stable when bombarded by electrons
    1. Contamination
    2. Decomposition and other alterations

Commercial instruments

Selected Techniques and Applications in Analytical Chemistry

Qualitative identifications
Factors involved in precipitation processes
Particle size determinations
Counting particles
Surface studies

Electrolyses at Controlled Potentials. L. B. ROGERS, Massachusetts Institute of Technology.

In an ordinary electrolysis, the electrode potential is usually limited primarily by gas evolution, although it changes somewhat with the magnitude of the current passing through the cell. Thus a separation depends upon one's ability to interpose gas evolution between the deposition potential for one element and those for the remaining elements.

Early attempts to separate elements without resorting to limitation of the potential by gas evolution employed anodes of various active metals, dissolution of which provided a characteristic potential. This method, however, had a number of serious drawbacks. Recently, advances in instrumentation have made automatic control possible, so that the probable number of applications have been increased. As yet, however, the number of published applications have been few.

The technique of electrolyzing at a controlled potential is useful not only for electrodeposition but in reactions producing soluble ions or compounds. Once again, the number of published applica-

tions is small. Some representative applications from inorganic and organic chemistry were mentioned.

Organic Reagents. LYNNE MERRITT, University of Indiana.

Formation and Stability of Chelate Compounds

A thorough, quantitative understanding of the factors that influence the formation and stability of chelate compounds is necessary if, in the future, the search for new and more selective organic reagents for inorganic ions is to be more efficient.

The following factors are probably of importance in the formation of chelate compounds:

Availability of acidic and basic groups in the organic molecule
Correct geometrical relationship of the two above-mentioned coordinating groups so as to form proper sized ring
Steric hindrance by neighboring groups
Availability of electron pairs of the coordinating groups for sharing with the inorganic ion—i.e., acid and base strength
Electron affinity of inorganic ion
Size of inorganic ion with respect to distance between coordinating groups
Spatial arrangement of coordinating groups and spatial requirements of inorganic ion
Solvent effects

Possibility of resonance in complex so that additional stability is gained thereby. Availability of necessary orbitals

The evidence for, and influence of, each of these factors in chelate formation were briefly reviewed.

Measurement of Stability and Analytical Applications of Knowledge

Methods of measuring the relative stability of various inner complex salts of analytical interest were discussed. These methods include measurements of solubility products, determination of the pH of incipient or complete precipitation, determination of the relative number of chelating anions bound by one cation as a function of pH, rate of exchange determinations, polarographic half-wave potential determinations, and other means.

Determinations of the crystal structure of analytically important compounds by x-ray diffraction means lead to much interesting information regarding the arrangement of atoms, bond lengths, etc., in these compounds. The structure determinations reported thus far in the literature were reviewed. Many interesting conclusions can be drawn from the space group determinations which are a preliminary phase of a crystal structure determination.

Several applications of the use of organic reagents in inorganic determinations which depend on a knowledge of relative stabilities were given. These include: titrations with end-point detection similar to the Mohr method for chloride ion, use of two reagents to isolate one ion selectively, and several incidental applications.

Colorimetric Analysis. GILBERT H. AYRES, University of Texas.

Fundamentals of Photometric Analysis

Utility of colorimetric methods
Laws of spectrophotometry
Methods of color comparison
Objective versus subjective methods
Instrumentation
  Sources of radiant energy
  Wave length selectors
  Photoreceptors
  Types of instruments
Method of study of a colorimetric method
  Spectral characteristics
  Reagent concentration
  Order of addition of reagents
  Rate of color formation
  Stability of color
  Temperature coefficient
  Effect of pH
  Range and accuracy
  Sensitivity
  Interferences
Determination of formula of colored product
  Method of continuous variation
  Molar ratio method
  Slope ratio method

Evaluation of Accuracy in Photometric Analysis

Historical background
Application of the laws of spectrophotometry
Methods of plotting photometric data
  Scale reading *vs.* concentration
  Beer's law (straight-line) plots
  Log absorbancy *vs.* concentration
  Ringbom method of plotting
The error function
Attainable accuracy
Optimum range
Methods of extending the range
  Choice of cell length
  Use of different wave lengths
  Measurement of transmittance ratios
Scope of absorption spectrometry

Chemical Oscillometry. OLEN A. NANCE, Louisiana State University.

Review of standard methods of electrical measurements
  Conductometric analysis
  Capacitance measurements and the dielectric constant
  Pertinent elements of standard circuitry
Oscillometry
  Review of published circuits and data
  Discussion of investigations of L.S.U.
  Suggested applications and suggestions for future developments in the field

The Analyst's Calendar

Pittsburgh Conference on Analytical Chemistry

The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy will be held in the William Penn Hotel, Pittsburgh, Pa., March 5 to 7, 1951. On the afternoon of March 5 a full session of the conference will be devoted to a testimonial to Keivin Burns, a pioneer in the field of emission spectroscopy, who is retiring after 30 years as astronomer at Allegheny Observatory of the University of Pittsburgh. The session will be composed of papers by S. C. Crawford, University of Pittsburgh; B. H. Carroll, Eastman Kodak Co.; N. E. Wagman, Allegheny Observatory; G. R. Harrison, Massachusetts Institute of Technology; and W. R. Brode and W. F. Meggers, National Bureau of Standards. A testimonial dinner will be held in the evening, at which W. F. Meggers will preside as toastmaster.

N. Howell Furman, president of the AMERICAN CHEMICAL SOCIETY, will be a keynote speaker. Walter J. Murphy, editor of ANALYTICAL CHEMISTRY, *Industrial and Engineering Chemistry*, and *Chemical and Engineering News*, will deliver introductory remarks. Other addresses will be delivered by M. G. Mellon, Purdue University, and G. H. Dieke, Johns Hopkins University.

A new feature of the conference will be an exhibition of books published during the past 5 years on analytical chemistry, spectroscopy, instrumental methods of analysis, and related subjects. As in past years, there will be an Exposition of Modern Laboratory Equipment at which about 20 exhibitors will present a complete assortment of instruments and apparatus for the analytical and spectrographic laboratory.

In response to requests, the conference will offer an employment service, with facilities for registration of employers and candidates for positions. Arrangements have been made for private conferences. Inquiries about this service should be directed to A. B. Steele, Mellon Institute of Industrial Research, Pittsburgh 13, Pa.

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. William Penn Hotel, Pittsburgh, Pa., March 5 to 7, 1951

Fourth Annual Summer Symposium. Washington, D. C., June 14 to 15, 1951

AIDS FOR THE ANALYST . . . .

A Constant Current Regulator. H. W. Patton, Vanderbilt University, Nashville, Tenn.

THE need for maintaining a constant direct current of about 1 ampere in a resistance heater has led to the development of a regulating device, which, when used in conjunction with lead storage cells, gives satisfactory compensation for small variations in current due to changes in circuit resistance or battery voltage. Except for the galvanometer and potentiometer, which are ordinarily available in a chemistry laboratory, the regulator was constructed from standard materials, the total cost of which was less than $10.

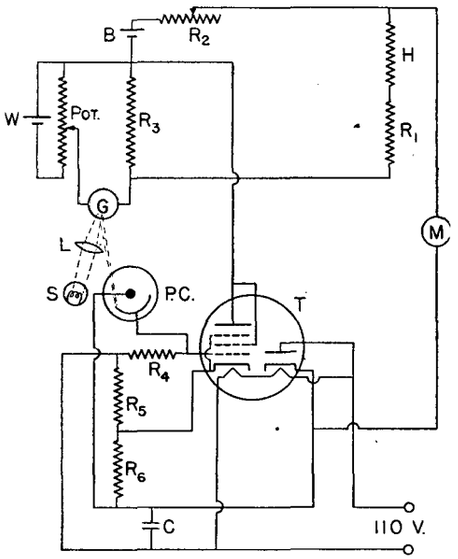


Figure 1. Heater and Regulator Circuit Diagram

B.	12-volt lead battery
C.	100-mfd., 150-volt capacitor
G.	Galvanometer
H.	Heater
L.	Lens
M.	Milliammeter
P.C.	Photocell
Pot.	Potentiometer
R_1.	Standard resistor, 1 ohm
R_2.	Rheostat, 30 ohms
R_3.	Control resistor, about 1 ohm
R_4.	2-megohm, 0.5-watt
R_5.	1200-ohm, 2-watt
R_6.	12,000-ohm, 2-watt
S.	Light source
T.	117L7-GT radio tube
W.	2-volt lead cell

Figure 1 is a schematic diagram of the heater circuit and current regulator.

The current flowing through the heater, *H*, consists of contributions from two sources. Almost all this current is furnished by the 12-volt batteries, *B*, but a small amount, which is automatically varied by the other elements of the regulator in such a manner that the total heater current remains constant, is furnished by the electron tube, *T*. The amount of this additional current depends on the intensity of light falling on the photocell, *P.C.* This light intensity in turn is controlled by the position of the mirror on galvanometer *G*, which reflects more or less light from the source, *S*, onto the photocell, depending on the deflection of the galvanometer. The deflection of the galvanometer is fixed initially by adjustment of the potentiometer, *Pot.*, which balances the potential across the control resistor against a part of that produced by the potentiometer working battery, *W*. After the initial setting is made, regulation of the desired current strength is completely automatic. If the current through the heater does decrease, owing to an increase in resistance of the heater circuit or decrease in battery voltage, the voltage across control resistor R_3 becomes smaller than that of the potentiometer, and the galvanometer is deflected in such a direction that more light is reflected from *S* onto *P.C.* The resulting increase in current supplied by the radio tube almost completely compensates for the initial decrease in heater current. A heater current larger than the desired amount causes a similar but opposite reaction of the various control elements, resulting in decreased current output from the radio tube. Hence in either case the total heater current remains essentially constant. These compensations are definite and almost instantaneous.

The range of variation in current which can be controlled by the device is limited by the maximum output of the radio tube. The tube used, Type 117L7-GT, has a maximum output of about 50 ma. Hence the control potentiometer could be adjusted so that the initial output of the radio tube is about 25 ma., and the device would successfully compensate for any changes in the heater circuit which caused the current supplied by the batteries either to increase or decrease by about 25 ma. The range of control could undoubtedly be extended by the use of a tube with a greater maximum current output, or by the use of two or more tubes in parallel.

The sensitivity of the device is affected by the nature of several of its components. Increased sensitivity can be accomplished by any one, or any combination of the following: use of a more intense light source, a more sensitive galvanometer, or a more sensitive photocell.

In the present setup, the light source consists of a single-element 6-volt incandescent lamp manufactured for use in automobile headlamps and rated at 30 candle power. The galvanometer is a Leeds and Northrup, Type R(2500-e). The photocell is manufactured by the General Electric Co. and bears the designation CL-868/PJ-23. An important part of the regulator is the lens, which is used to focus light from the source onto the galvanometer mirror in such a manner that the cross section of the beam as it reaches the photocell is circular and about 2 mm. in diameter. This allows a very slight movement of the galvanometer coil to vary the intensity of light falling on the photocell from practically zero to the maximum available. It is possible that greater sensitivity might be obtained by the use of a needle-type galvanometer fitted with an opaque vane similar to that used by Longsworth and MacInnes [*J. Optical Soc. Am.*, 19, 50 (1929)] in their regulator.

That part of the heater current supplied by the radio tube is, in the setup shown, rectified and filtered 60-cycle alternating current. By means of a cathode ray oscilloscope, the "ripple" voltage was estimated to be less than 0.5% of the total plate voltage. Because the control current supplied by the tube is only a small fraction of the heater current, the error introduced due to the alternating current component is negligible in the present work. The use of a better filtered power supply, or a battery source, for plate voltage might be desirable for certain applications.

When the regulator is connected directly to line current as shown, the heater circuit wiring is a possible source of electrical shock, and should be handled with care. This feature can be removed by the use of an isolation transformer.

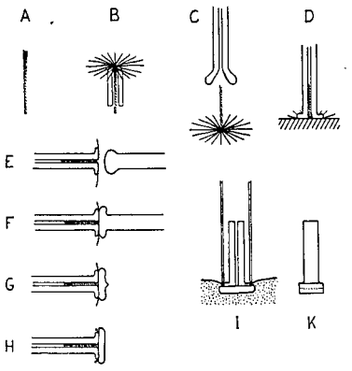
Maintenance of a constant current by this device is dependent on the constancy of both the voltage of the potentiometer working battery, *W*, and the resistance of resistor R_3. In the regulator described, the working battery is a 100-ampere-hour lead storage cell, which is kept connected to the potentiometer at all times. For operation over long periods of time, the change in working battery voltage might necessitate periodic readjustment of the potentiom-

eter. R_3 is wound from Manganin wire and immersed in oil. The degree of regulation made possible by the device is demonstrated by the fact that during the course of 30-minute runs, the variation in current has never been found to exceed 1 part in 25,000.

In addition to applications in which precise energy measurements are to be made, the regulator is useful for maintaining constant currents during the calibration of resistances and direct current meters. It seems possible that for the maintenance of constant current during moving boundary and electrophoresis experiments, a modified regulator based on the principle described might be superior to the one used by MacInnes and Longsworth [*Chem. Rev.*, **11**, 189 (1932)].

Preparation of Bubbler Tips. Charles L. Gordon, National Bureau of Standards, Washington 25, D. C.

THE glass bubbler tips described by Branham and Sperling [*J. Research Natl. Bur. Standards*, **22**, 701–5 (1939)] are convenient and efficient for dispersing a gas into an absorbing solution. They are made by sealing a number of fine copper wires between a cone formed on the end of a glass tube and a circular glass disk. The ends of the wires are then exposed by grinding and the wires are dissolved in acid. Their construction is rugged, yet their availability has been limited because of the difficulty of manufacture. A less exacting method of manufacture is given here.



Method of Construction. Prepare a spider of copper or palladium wires of the size chosen for the holes by twisting the desired number of wires together so that about 0.5 inch (1.25 cm.) of untwisted wire remains as illustrated at *A*. Clip off the twisted portion to reduce the total length to about 1.5 inches. Hold the wires over a flame momentarily to anneal them, then insert the twisted end into a jig and clamp it. Splay out the untwisted wires, as shown at *B*, to radiate uniformly. Uniform arrangement is best done by slotting the jig, but arrangement by eye gives a satisfactory bubbler.

Select a short length (about 2 inches) of capillary tubing of such bore that it will easily pass over the twisted wires and yet not be over twice their combined diameter. Heat one end of this tube in an oxygas flame and flare the opening to 5 to 6 times the diameter of the twisted portion of wires. Place the inverted spider on a sheet of brass near the flame. Heat the flared end of the tube until it is very soft, then quickly push it down over the stem of the spider onto the fan of wires, as at *C*. If the glass was hot and soft enough (and not too large in internal diameter), the spider of wires will adhere to it, as at *D*, on lifting the tube. Keep this assembly warm by holding in the warm blast far out beyond the end of the flame.

Heat one end of a glass rod until a soft ball of glass forms and becomes very fluid at the outer surface. Line up the tube held in one hand with the rod in the other and immediately press them together, as at *E*. The soft glass unites with the warm exposed glass between the spider wires, as at *F*. Soften the rod back of the flared head and draw it off, as at *G*. Soften and paddle (or marver) the remaining knob of glass to form a flat end over the spider, as at *H*, and anneal the whole end in a soft yellow flame.

Finish the bubbler tip by removing the excess peripheral glass. The simplest way is to rotate the tip against a high-speed Carborundum wheel. If the tip is held lightly, and the grinding is not hurried, this method yields a satisfactory tip in a minimum of time. This rough-ground tip may then be fine-ground by lapping it in a rotating tapered cylinder into which are fed water and emery powder, as at *Q*.

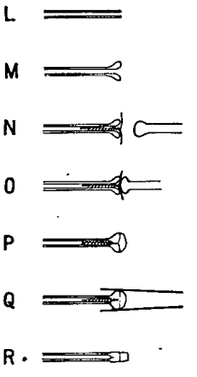
Second Method. Lap out the desired portion of the tip from the excess glass on the head of the tip by means of a rotating slotted tube, mounted in a drill press, to which are fed water and emery. Cement the tip by plaster of Paris to a block of wood and clamp it to the table of the drill press. For a lap use a piece of brass tubing of appropriate inside diameter to reduce the tip to the diameter desired. Run the rotating lap tube, slotted at the cutting end to admit the grinding compound to the cutting edge, down over the stem of the bubbler tip and continue lapping, *I*, until the tip is left, as at *K*.

Finally polish the tip, if desired, and dissolve the wires with warm nitric acid.

Tips made in this manner can be finished to the same diameter as the capillary tubing to enter any apparatus into which the tubing alone can be inserted. For very small tips the smaller area of the seal in the glass between the wires of the spider requires a more positive method of fusing the glass around the wires, and platinum or palladium is more appropriate for the spider. For holding and spreading the spider wires, it is convenient to solder them together with gold at the spreading point.

After proceeding as to *H* (*L* to *P*) fuse the whole end of the tip until it is nearly spherical, as at *P*. Then rough-grind the tip to a slight cone as at *R* by rotating against a high-speed Carborundum wheel. (This method, while quick, sometimes breaks the glass.) Then lap with emery in a slightly tapered tube as at *Q* until the diameter has been reduced to the desired size.

The spiders made of palladium have a tendency to be surrounded by bubbles in the glass, caused by gas escaping from the wire at the higher temperature of fusion. The capillary tubes formed by platinum wire are neater, but a longer time is required to dissolve the wires with aqua regia (4 parts of hydrochloric acid, 1 part of nitric acid, and 1 part of water). The time for dissolving the wires is not unduly prolonged unless the wire has been hardened with iridium. In this case the tip can be sealed in a tube of borosilicate glass together with the acid mixture (without the 1 part of water) and heated at 110° C.



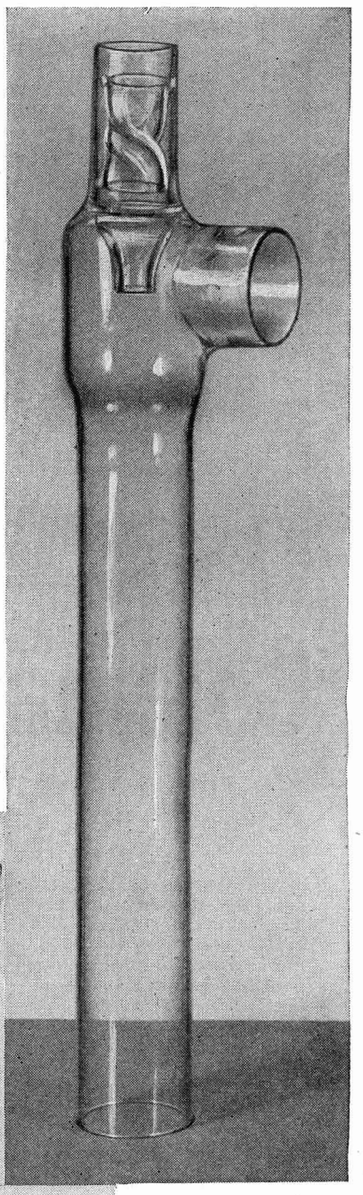
Tips with 20 and 30 openings formed by No. 44 B.&S. wire gage have been made using tubing of 3-mm. outside diameter.

Performance. Flow tests were made on a 9-mm. tip made from borosilicate glass capillary tubing of 6-mm. outside and 1-mm. inside diameter using 20 strands of No. 36 B.&S. gage copper wire. A pressure of 24 cm. of water was required to start the flow of air through the tip when immersed in water at room temperature. With a pressure of 35 cm. of water, air flowed through all the openings at a rate of 2.2 ml. per second. Above this the rate of flow was a linear function of the logarithm of the pressure up to about 4.5 ml. per second at a pressure of 52 cm. of water.

The main difference in the delivery characteristics between the example used here and the Branham-Sperling tips is in the length of the capillaries produced by the wire. The Branham-Sperling tips are, in general, more uniform and the length of embedded wire is shorter. The pressure required to bubble gas through the tip made as described here is, therefore, greater than those made previously. This can be decreased by grinding the periphery away to produce a shorter restricted passage. With care a bubble can be blown around the center of the spider to decrease the restricting path and increase diameter of tip head.

Sargent
EASY FILLING MANOMETER

Sargent
LARGE VOLUME ASPIRATOR



The Sargent Manometer combines the convenience of manipulation of the standard closed form with the simplicity of filling of the open type.

The manometer is a U-tube with a fine porosity fritted disc in one arm near the top. To fill, mercury is added to the open arm. Air is exhausted from the arm containing the fritted disc. Placed on top of the fritted disc is a shallow layer of mercury to prevent the return of air into the arm. The mercury will not pass through the disc under full pressure and maintenance of the necessary vacuum is thus assured. A constriction at the base minimizes oscillation and protects the disc from impact of the mercury column.

Total height, 290 mm; diameter of fritted disc, 10 mm; length of side arms, 35 mm.

The manometer is mounted on a finished wood stand provided with sliding scale graduated in millimeters to facilitate reading of level differences.

S-39742 GAUGE—Vacuum, Mercury, Closed Manometer Form, Fritted Disc Seal, PYREX Glass, Sargent. With stand, without mercury. Each.....**$20.50**

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● ● ● ● ●

Sargent's low pressure, large volume aspirator, made of Pyrex glass, produces a free air velocity at the intake of 500 feet per minute under water pressure of approximately 30 to 35 pounds per square inch. Under these conditions air is transferred at a rate of 20 cubic feet per minute.

If applied to a closed system the maximum pressure that this pump develops is about 5 inches of water column. It may thus be used to exhaust a volume of vapor from a closed system in which the development of substantial negative pressure is not desired. When applied to the exhaustion of closed systems it provides satisfactory disposal of large volumes of fumes which are readily soluble or readily condensable, including the vapors of acids, ammonia, etc. It may be used for drying in a current of air at low temperatures and for Kjeldahl digestions, etc.

This large volume aspirator measures 10 inches high. The water intake is ¾ inch in diameter, the exhaust is 1 inch in diameter, and the air intake will accommodate ¾ inch I.D. tubing.

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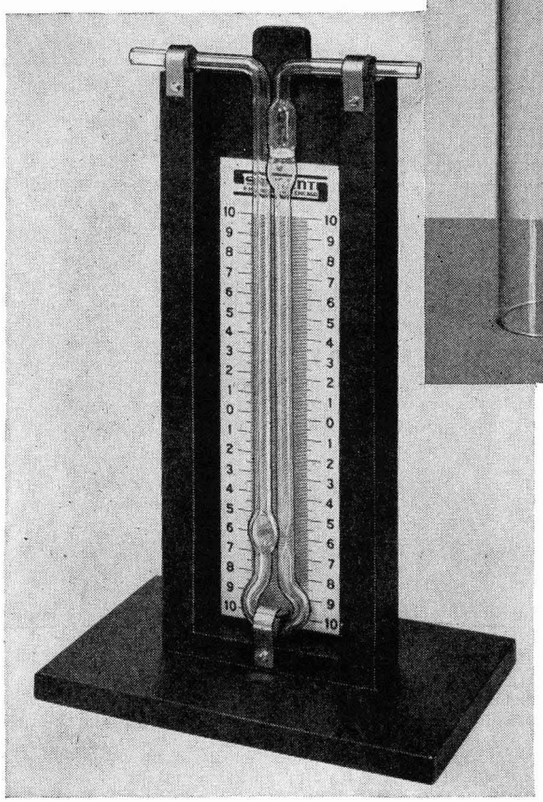

S-33650

S-39742

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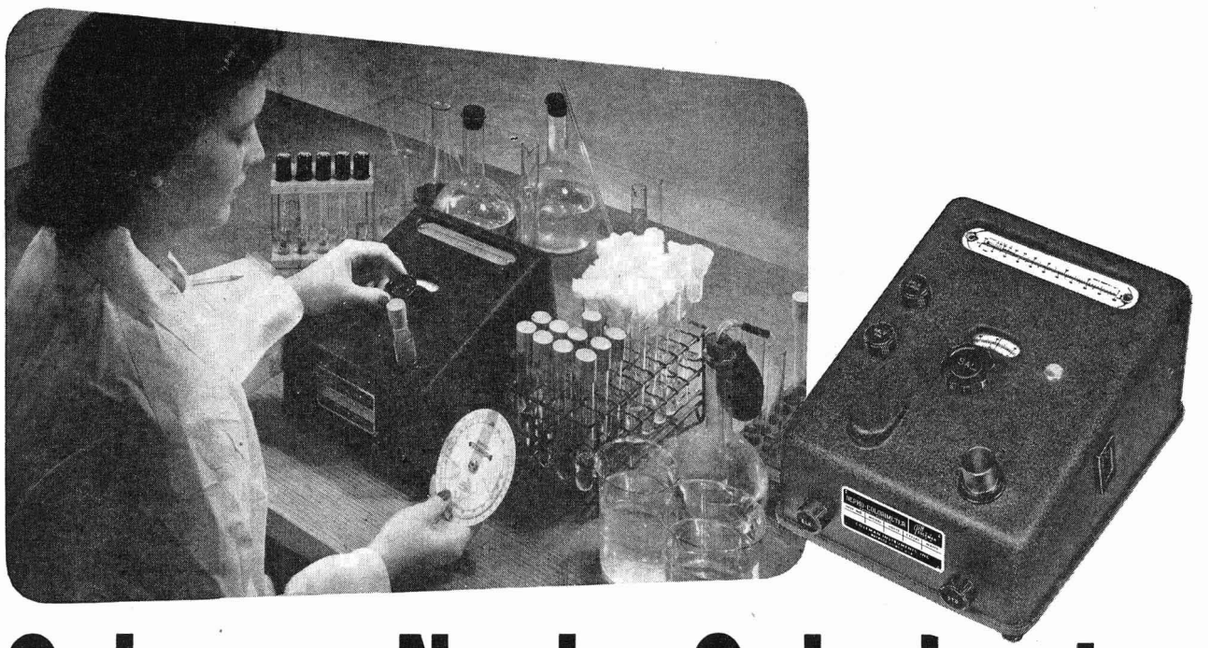

Coleman Nepho-Colorimeter

FOR THE EXACT MEASUREMENT OF COLOR, HAZE AND TURBIDITY IN LIQUIDS

FOR NEPHELOMETRY

Nephelometry is the science of evaluating haze in liquids by measuring its ability to reflect light, this ability being directly related to the concentration of the suspended phase. The Model 9 Nepho-Colorimeter is an extremely sensitive and highly precise instrument for measuring this reflected (Tyndall) light. Coleman Certified Nephelos Standards permit expression of such measurements in terms of a fixed, numerical scale. Data thus becomes universally significant and comparable. Repeated preparation of temporary standards is eliminated. The linear relation between instrument response and Tyndall light intensity makes Nephelos/Concentration calibrations simple and exact. Nephelometry is finding increasing application in the examination of Biologicals, Bacterial Suspensions, Industrial Waters, Beverages and Blood Sera.

FOR COLORIMETRY

Actuation of a single switch converts the Model 9 Nepho-Colorimeter from a nephelometer to a precision colorimeter capable of colorimetric measurements of a precision and certainty far above that ordinarily available in filter instruments. Selected filters cover the visible spectrum in six uniform increments. Scratch-proof mountings assure continued uniformity. Direct-reading scales for optical density or percent transmittance readings may be interchanged with scales individually calibrated for specific analytical methods. Interchangeable adapters accept cuvettes ranging from 25mm depth down to capillaries requiring only 0.01ml of solution.

FOR TURBIDIMETRY

When the particles in a liquid suspension are sufficiently concentrated to interfere substantially with the transmission of light, the amount of suspended material may be measured by determining the transmittance of the suspension and relating it to particle concentration. Turbidity measurements with the Model 9 Nepho-Colorimeter are characterized by the ease and speed of direct-deflection reading and by the unparalleled precision available from the calibrated potentiometer. Determinations can be made with either white light or colored light.

Write for Bulletin AB-215 for full information.

Coleman Photo-Nephelometer (MODEL 7)

For Nephelometry only. The Model 7 Photo-Nephelometer is capable of the same precision in nephelometric measurements as the Model 9 Nepho-Colorimeter but is not equipped for Colorimetric or Turbidimetric measurements by transmitted light.

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INSTRUMENTATION

Characteristic properties and uses of Thyrite nonohmic electrical elements are reviewed, with suggestions of applications in analytical investigations

by R. H. Müller

On numerous occasions we have discussed nonohmic electrical elements for the reason that many of them offer new and unusual applications, either as primary elements, or as means for measurement and control. In this class are thermistors, transistors, phototransistors, and the germanium crystal rectifier. Thyrite resistors are older than any of these, but apparently their measuring and instrumental possibilities have been neglected or overlooked, at least compared with their rather extensive industrial use. A recent article by R. P. Turner [*Radio and Television News*, **45,** 50 (January 1951)] reviews some of their characteristic properties and uses. We wish to draw the analyst's attention to this interesting article and to suggest ways in which this information may be useful in his work.

Thyrite Resistors

Thyrite is the trade name for a material developed by the General Electric Co. It is a ceramiclike mass consisting of silicon carbide and a ceramic mixed in various proportions and fired at a temperature of about 1200° C. The electrical units employing this material consist of disks to which flat electrodes are applied, or rods with pigtail leads. Both varieties are available in various sizes and power ratings and they are impregnated against humidity effects. Electrically, their behavior is nonohmic —that is, the current passing through the element is not directly proportional to the applied potential. The relationship is expressed by an equation of the form $I = kE^n$. The exponent n varies between 3.5 and 7.0, depending upon the manufacturing technique. Although k is a proportionality constant, it is evaluated for practical purposes in amperes at 1 volt applied. A Thyrite resistor differs from some of the other nonohmic devices in that its action is almost exactly symmetric. According to G.E., rectification effects are less than 1%. This is most readily shown by applying a.c. to the device and displaying the resultant current on an oscillograph. If the resultant current is applied (as an RI drop) to the vertical plates of the oscillograph and the applied potential to the horizontal plates, a symmetrical pattern results, showing the rapid exponential rise in the first quadrant and equal exponential downward deflection in the third quadrant.

As might be expected from the nonlinear behavior, the Thyrite resistor is a good harmonic generator—that is, if a sinusoidal potential is applied to it, the resultant current will contain harmonics of the fundamental frequency. The odd harmonics are favored. In some applications this harmonic distortion is objectionable and must be considered. On the other hand, it is a convenient means for frequency multiplication.

Turner quotes the General Electric Co. Bulletin G.E.A.-4138B for typical Thyrite characteristics, among which may be noted that Thyrite characteristics do not change with time and that they are immune to pressure or vibration effects. They will respond to d.c. or to transients as short as 1 microsecond. There are no polarization effects. The temperature coefficient of resistance is negative and at constant voltage it varies between −0.4 and −0.73% over the range 0° to 100° C. The average Thyrite resistor can dissipate about 0.25 watt per square inch continuously. For short-time tests or under transient conditions and assuming no time for radiation, the temperature will rise 80° C. for 2000 watt-seconds per cubic inch of Thyrite. To avoid electrode oxidation effects, continuous temperatures in excess of 110° C. should be avoided, or for short intervals temperatures in excess of 150° C. Although the impregnation of units renders them immune to humidity effects, whenever currents of less than 1 ma. are measured, or when very high humidity prevails, special precautions may be required to minimize these effects.

Heretofore, the Thyrite resistor has found extensive use as a two-terminal voltage regulator, as a protector against voltage surges, as a lightning arrestor, as a sensitive element in voltage detector circuits, and for providing constant voltage output for varying loads. In consideration of its unique characteristics, and the fact that it is equally useful for a.c. or d.c., it may be seen that it has great potentialities in other applications.

Use in Voltage-Sensitive Bridge

Let us consider its use in a voltage-sensitive bridge with reversible output. If two Thyrite resistors and two ordinary resistors are connected in simple bridge fashion, and such that the Thyrites are in diagonally opposite arms, then there is one, and only one applied voltage at which the bridge will be in balance, and for which the output of the bridge is zero. If the potential applied to the bridge is d.c. and its value is increased above the balance value, the output will no longer be zero, and the output terminals will have a definite polarity. Conversely, if the applied voltage is decreased, the bridge will be unbalanced again, but the output polarity will be reversed. Similar results

INSTRUMENTATION

have been obtained by using incandescent lamps for two arms of a bridge, but their range is much more limited and they are likely to be much more sluggish in response.

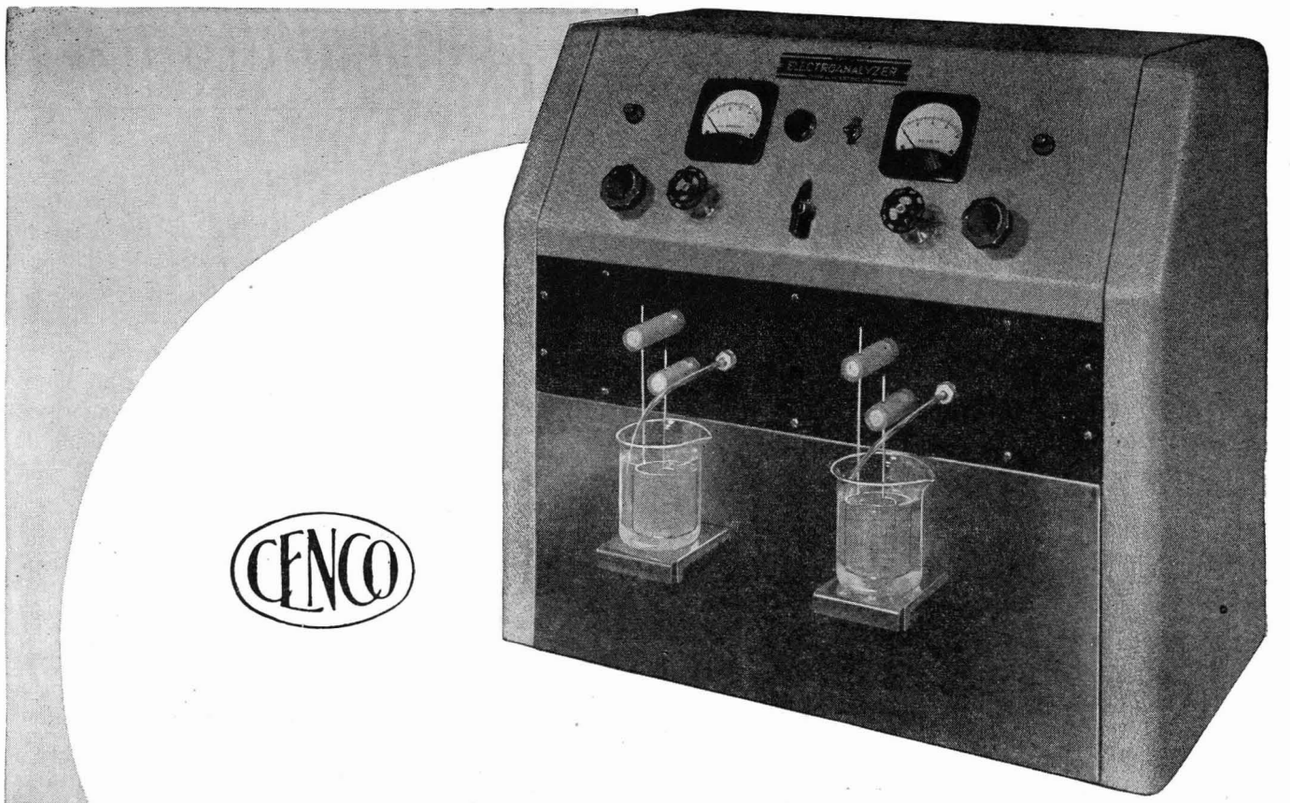
If a number of Thyrite resistors are connected in series and the current through them is derived from a varying source, the potential drops across the Thyrite elements will be sensibly constant because their resistance decreases with increasing current. This affords a voltage divider giving several constant voltage outputs.

As an output voltage regulator for either a.c. or d.c., an ordinary resistor and a Thyrite resistor are connected in series to the unregulated source. The regulated voltage is obtained from the drop across the Thyrite resistor. It is obvious that power losses will be incurred in this procedure and must be taken into account. The degree of regulation can be improved, at the expense of additional losses, by cascading several stages of this sort.

The exact inverse of this process can be achieved if it is desired to augment or overemphasize voltage variations. Once more, two resistors are connected in series with the source, one an ordinary resistor, the other Thyrite. The output voltage is now the drop across the ordinary resistor. This effect can also be cascaded to produce large variations. It can be seen that this scheme affords an extremely compact and simple system for the exaggeration of small unbalance e.m.f's. wherever those may be used effect the rebalance of an a.c. or d.c. system.

In electronic circuitry, Thyrite resistors have distinctive uses. If one of them is used in the cathode lead of a tube, it will provide an essentially constant bias voltage for the tube. Constant output of a tube can be attained if a Thyrite resistor is used as the cathode resistor in a cathode-follower circuit.

(Continued on page 26 A)



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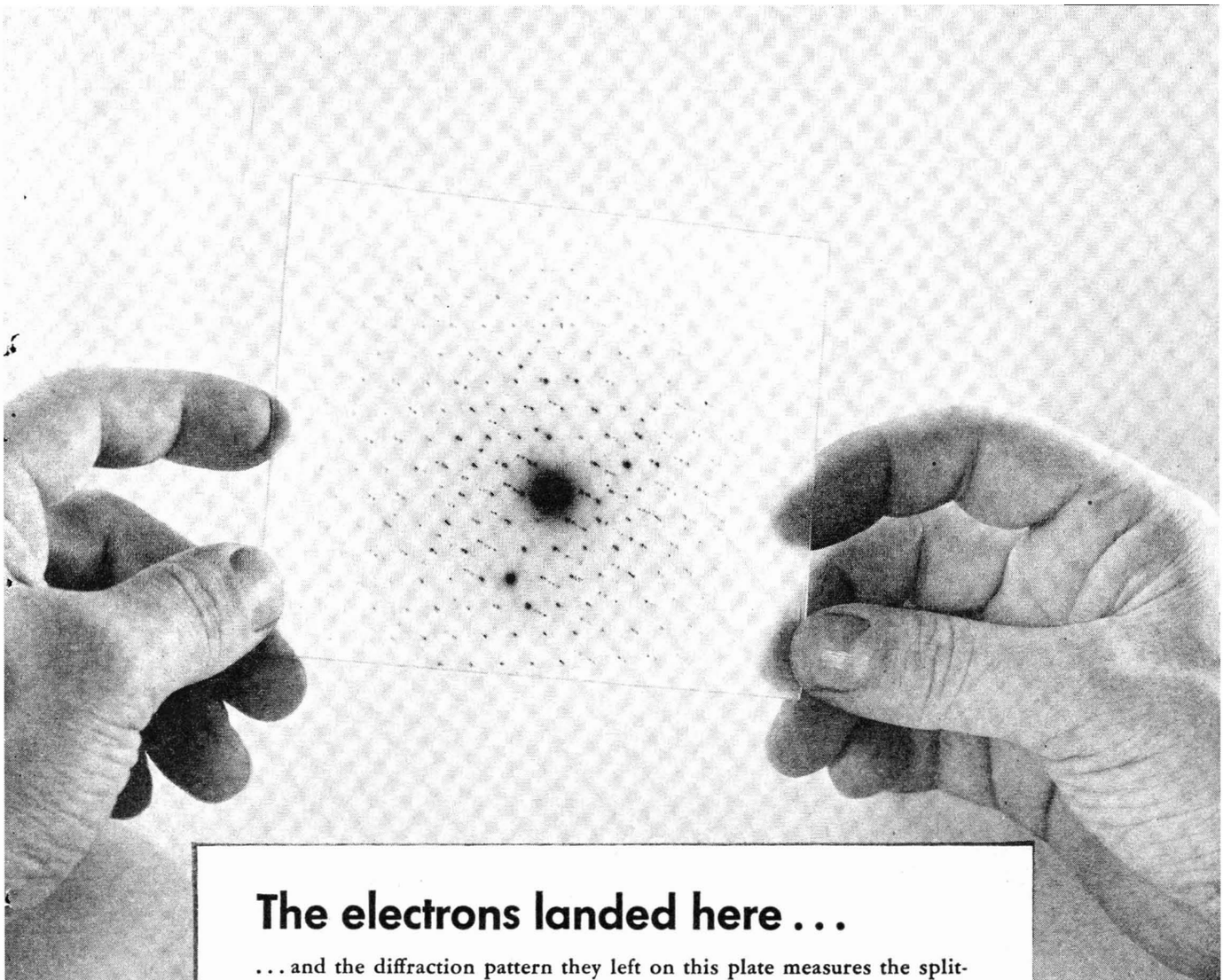
INSTRUMENTATION

It is one of the characteristics of the present age that great attention is being given to the development and study of new devices. There is no end to the degree of elaboration which can be made of current theory and practice, but it seems that these developments in the newer crystal physics—the characteristics and properties of semiconductors and nonohmic system—will continue to supply us with extremely useful devices.

Computational Aids

Although there are much acclaim and publicity about digital computers and the prodigious mathematical feats which they can accomplish, the chemist is still in need of relatively simple computational aids. This is evident if one examines the very simplest expressions involving chemical phenomena. We are constantly confronted by expressions involving squares, roots, logarithms, and reciprocals. To the extent that more of our analytical procedures are yielding to instrumental solution, we are confronted with the necessity of eliminating the residual chore of computation. This cannot be met by a computer which costs several thousand dollars. But to the extent that a simple element can indicate a logarithm or extract a square root, this may be added to an instrument at small cost and with great profit. In passing, it may be noted that elements like the Thyrite resistor and its relatives show promise in this direction. Computationwise, the promise resides in its nonlinear behavior. One could devise countless circuit combinations to match a desired mathematical function or produce an acceptable approximation. The actual solution is best achieved by an applied mathematician or computer expert, but his needs must be suggested by the chemist. One may expect to see considerable progress in this direction in the near future.



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For industry, electron diffraction patterns are revealing and identifying oxides on non-ferrous metals, the faint beginnings of corrosion on steel, scale in distillation equipment, crystal formations in chemical processes—thin films of all kinds.

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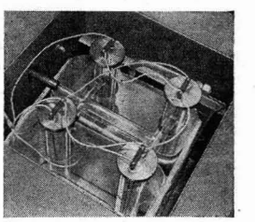
The Aloe modern stainless steel Freezing-Dehydration Apparatus is designed on the basic principles first outlined by Richard Altmann and more recently modified by Isadore Gersh. Although the original Aloe Freezing-Dehydration Apparatus has proved entirely satisfactory for many problems, this improved unit incorporates these important new features: For added convenience in handling the specimen, the vacuum chambers are now equipped to permit direct embedding. Both temperature and vacuum are accurately shown on direct reading gages. Troublesome glass joints have been replaced by improved flexible vibration proof joints.

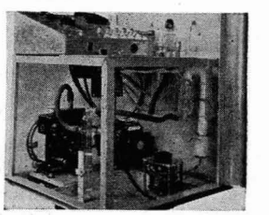
Under accurate controls, the new Aloe Freezing-Dehydration Apparatus permits tissue to be dehydrated and fixed with minimal losses and alterations. Tissue, after preliminary freezing in isopentane or liquid air, is placed in one of the vacuum chambers in the refrigerated compartment. Temperature is adjusted from 0° C. to —40° C. and vacuum to 0.001 mm mercury. If desired, the specimen may be embedded directly without breaking the vacuum. Embedding may also be carried out in some inert atmosphere such as nitrogen. Sections of embedded materials may be used for morphological and cytochemical studies by numerous methods.

JL71580—Freezing-Dehydration Apparatus, complete with vacuum-dehydration assembly, mechanical and mercury diffusion pumps, resistance thermometer, precalibrated vacuum gage, specimen containers, and built-in embedding system. Each.....$2,675.00

Control Panel includes gages for vacuum or refrigeration.



Refrigeration Compartment specimen assembly with caps.



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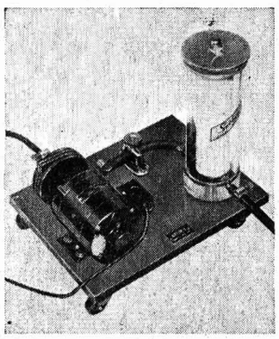
Low Temperature Refrigerator

The Collins helium cryostat, a self-contained, low-temperature refrigerator, is available from Arthur D. Little, Inc. The unit can maintain any temperature down to −270° C. without auxiliary refrigerants. The apparatus permits the liquefaction of either helium or hydrogen at a rate of 4 liters per hour if liquid nitrogen is used for precooling and 2 liters per hour without precooling. The compressor, driven by a 15-hp. motor, operates in four stages and delivers helium at a pressure of 225 pounds per square inch. The over-all dimensions of the compressor are 7.5 × 3.5 × 3.5 feet, and it weighs 1100 pounds. **1**

Filter Pump

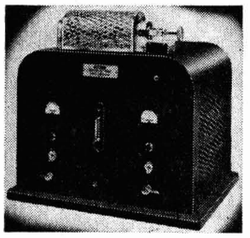


Sethco announces a new model filter pump known as Model LSI-10. Rated at 100 gallons per hour, the unit is equipped with a high-temperature Lucite filter cylinder and a stainless steel pump. The filter pump is highly resistant to all electroplating and industrial solutions. Liquids up to 200° F. may be used. The filter cylinder holds less than 1 quart of solution, every drop of which, says the company announcement, may be recovered. The filter element is a specially processed cotton yarn which is wound around a stainless steel supporting core. The progress of the filtration may be watched through the transparent Lucite cylinder. **2**

Extra-Large Tongs

For those who work around laboratory furnaces, a product has been developed by Fisher Scientific Co. for the safe handling of sample crucibles. With the new tongs, no longer must the operator's hand be exposed to extreme furnace heat. The tongs are nearly 21 inches long and are made of 18-8 stainless steel. Positive control of crucibles is assured by a patented handle which provides separate grips for the thumb and one finger. The hand firmly supports the entire weight of the crucible and tongs because the handle extends well beyond finger grips. Selling price, $3.50 each or $37.80 per dozen. **3**

Carbon Determination



The Burrell Corp. has announced a new electronic instrument known as the Combustron, which makes possible the rapid and accurate determination of carbon by combustion. It is a compact, bench-mounted, self-contained instrument which comes fully equipped and ready to plug into an electrical outlet. This device employs induction heating and incorporates such features as instant heating, visible combustion, a sturdy Vycor reaction tube, and availability in one- or two-tube models. It requires 115 or 230 volts and a 60-cycle, single-phase power supply. **4**

Freeze Dryer

The Model 203-L freeze drying unit, manufactured by F. J. Stokes Machine Co., has a rated capacity of 3.6 liters. It can be used for general research purposes and in the small-scale production of guinea pig complement, cultures, serums, vitamins, and other biologicals. Drying and freezing are done in a tank at the top of the chamber on an electrically heated and thermostatically controlled drying shelf. The equipment includes a Stokes McLeod vacuum gage and Stokes No. 146 high vacuum pump. A stainless steel dry ice condenser finger prevents water vapor from reaching the vacuum pump. Freezing is observed through a sight glass in the lid of the freezing unit. Model 203-L can also be supplied with chemical desiccant in place of the dry ice equipment, or with both. **5**

Ultrasonic Generators

A newly developed line of ultrasonic generators for biological, physical, and chemical research has been reported by Columbia Technical Corp. Ultrasonic energy at a frequency

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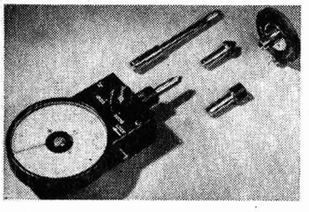
of 800 kc. is produced by means of a piezoelectric crystal. The acoustic power output can be varied from 0 to 5 watts per square centimeter and accurately maintained at any intensity level. The unit is simple to operate in view of its compact construction. It may be connected to a standard 115-volt a.c. wall socket. The sound emitter is made of chromium-plated steel and completely sealed. It can be used for applications under water and in noncorrosive liquids. Three sizes of emitters with acoustic power outputs of 25, 35, and 50 watts are available. **6**

Hand Tachometer

The new Smiths Model A.T.H. 10 dual-range hand tachometer features extremely low torque—0.40 ounce-inch on the lowest range. The ranges are 0 to 1000 and 0 to 5000 r.p.m. Each range is printed on the dial in a contrasting color. The revolving Alnico permanent magnet inside the drag cup assures high sensitivity. Offered by Equipoise Controls, Inc., the instrument has a guaranteed accuracy of 0.5% over the entire scale range. The device is provided with a knurled knob for range selection and a push button on either side for releasing and/or holding the pointer at the machine speed indication. Packed in a velvet-lined Fabricoid carrying case, the tachometer is complete with male and female centers, 3.5-inch extension, and 6-inch circumference surface measuring disk. **7**



β-Glucuronidase

The Sigma Chemical Co. is producing bacterial β-glucuronidase. The supplier states that the enzyme will quantitatively hydrolyze phenolphthalein glucuronide in a very short time and at pH 7.0, whereas the optimum pH for mammalian glucuronidase is 4.5. β-Glucuronidase is available as a prepared solution (pH 7.0) or as a dry powder which can be reconstituted with distilled water to yield a buffered solution directly. Its stability is good. **8**

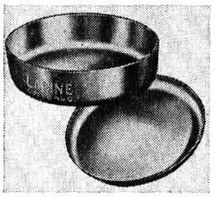
Crystalline and Plastic Phosphors

Tracerlab has available two phosphorescent materials for use in scintillation detector units. One is the synthetically grown hydrocarbon crystal known as stilbene and the other is a solid plastic phosphor. Stilbene is useful as a universal phosphor standard for scintillation counter work because it is nonvolatile and is stable despite fluctuations in the moisture content of the atmosphere. Precisely formed phosphors of up to 1 inch in any dimension are readily machined from the colorless, optically clear, single crystal masses of stilbene. The violet light emitted by this phosphor has the required intensity to operate modern counting equipment with high efficiency. The time constant of stilbene, less than 10^{-8} second, is less dependent upon temperature than are most other phosphors.

While most requirements for luminescent material can be met by phosphors which are no greater than 1 inch in any dimension, high efficiency gamma counting, cosmic ray research, and fast neutron counting require considerably larger phosphors. The cost of crystalline phosphors of the necessary size would be prohibitive. Consequently, Tracerlab has developed an inexpensive luminescent plastic which is easily molded into a large variety of shapes. It can be molded so as to embed two 1P21 photomultiplier tubes operating in coincidence to reduce multiplier noise background. The phosphor pulses of this plastic are about one quarter as large as those of stilbene when viewed with the S9 spectral response of a 5819 tube. Pulse duration is of the same order of magnitude as that of the organic crystalline phosphor—less than 10^{-8} second. **9**

Stainless Steel Dishes

Arthur S. LaPine and Co. offers stainless steel dishes suitable for use in weighing, crystallization, evaporation, milk analysis, and grease stability tests. The large dish is 3.125 inches in diameter and 0.75 inch in height, capable of holding approximately 40 grams. The smaller size is 3 inches in diameter and 0.25 inch in height and holds approximately 21 grams. The dishes are of one-piece construction. Employing 18-8 stainless steel, they are resistant to most reagents. Their rounded bottom edge facilitates the removal of foreign matter. Minimum trial order: 3 large dishes for $2.75 or 3 small dishes for $2.00. **10**



Pyrometer Equipment

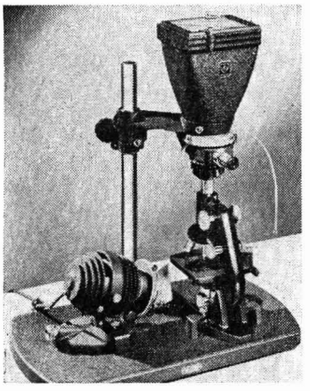
New pyrometer equipment which offers accurate temperature indications, close temperature control of industrial processes, and protection of furnaces, ovens, and kilns has been announced by General Electric's Meter and Instrument Divisions. The complete line consists of flush- or surface-mounted indicators, controllers, and protectors. The instrument has a calibrated accuracy within 0.75% of full scale. A legible 7-inch scale, fitted with an antiglare cover, indicates

any change in temperature equivalent to 0.1% of full scale. Immediate control action follows. Normal changes in humidity, ambient temperature, and voltage have little or no effect on the exactness of the control action.

The heart of the indicating instrument is a 3.25-pound magnet, which provides higher flux density and allows larger air gaps than are found in conventional instruments. The indicating device is a millivoltmeter connected to a thermocouple on the furnace or other heating equipment. The Type HP-3 controller provides on-off action of the final control element by a relay, mercury switch, or contactor through which electric power is supplied to the furnace or oven. The pyrometer protector, a separate form of the HP-3, is usually used in conjunction with and to protect against possible failure of a separate precision controller. **11**

Photomicrograph Cameras

A new series of AO Spencer 35-mm. and 4 × 5 inch photomicrograph cameras has been announced by American Optical Co. Designed to replace a line of cameras discontinued during World War II, these cameras have a unique revolving body feature which permits 360° rotation of the camera backs. This eliminates the need for a microscope with an expensive circular revolving stage. A light-tight adapter permits the photographer to swing the camera over or away from the microscope without the need for raising or lowering the camera body or disturbing the focus of the microscope. A fine-adjustment stop on the camera arm assures precise repeat positioning and thus faster operation. Critically sharp focusing is obtained with a telescopic focusing eyepiece equipped with cross-hair reticule. The image may be viewed in the telescope up to the moment when the shutter is released. The 4 × 5 inch ground glass has diagonal transparent strips across the field which enable a magnifier to be used to check the quality of the image when especially critical focusing over the entire plate arm is essential.



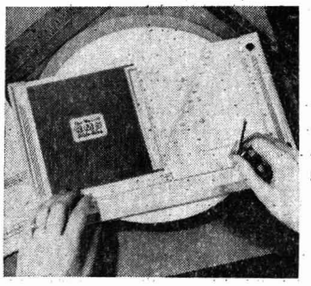
Three models are offered: 4 × 5 inch camera with Universal shutter and telescopic focusing eyepiece; 35-mm. film camera with Universal shutter and telescopic focusing eyepiece; and 4 × 5 inch camera with Alphax shutter. The 35-mm. camera is interchangeable with the 4 × 5 inch body and is equipped with a compensating lens to accommodate for the difference in focal length between the two bodies. Holders are available for 4 × 5 inch plates, films, or film packs. **12**

Abbe Refractometer

A new Model G Zeiss Abbe refractometer has been announced by the Scientific Instruments Division of Ercona Corp., American representative for Carl Zeiss Jena products. While preserving the standard Abbe principle, the Zeiss works have incorporated such new features as a stationary reading microscope in place of the former movable magnifier. The telescope for observing the border line and the reading microscope are located side by side in an inclined position for maximum convenience and speed. This arrangement permits one to check the border line with the right eye and to read the index value with the left eye without changing position. The operating drum for setting the border line is placed in a low position, so that the operator's hand rests comfortably on the table during manipulation. The graduated glass circle carries two scales for n_D values 1.3 to 1.7 and for dry solids, 0 to 85%. The instrument permits readings of 1 to 2 units in the fourth decimal of the refractive index and 0.1 to 0.2% in the case of dry solids. **13**

Graphic Calculator

Statisticians in industrial quality control will welcome a new desk calculating instrument, the Merrill RMS slide-disk, which simplifies the computation of standard deviations, root-mean-squares, and correlation coefficients. By a special principle of graphic calculation, statistical results are obtained in a fraction of the time previously required and to an accuracy of 0.5% or better. Time savings result from the fact that as many as five mathematical steps are performed in one simple mechanical operation taking about 2 seconds to complete. The RMS slide-disk consists of a 10-inch disk that slides and rotates under a pair of vertical and horizontal scales on which a series of right triangles is formed graphically. The instrument makes use of the Pythagorean theorem (the hypotenuse of a right triangle is the square root of the sum of the squares of the sides). The operations of squaring, summing squares, and square rooting so commonly required in statistical computations are performed simultaneously and with the ease and rapidity that come from graphical solution. The slide-disks are furnished with linear and logarithmic scales and an instruction manual. All critical parts are made of dimensionally stable Vinylite plastic. Offered by the Graphic Calculator Co., the instrument sells for $64. **14**



Battery Eliminator and Charger

The Electronic Instrument Co., Inc., is now manufacturing a new Model 1040-K battery eliminator and charger kit. It features a full-wave bridge circuit consisting of 4 heavy-duty manganese-copper oxide rectifiers. Its transformer is variable from 0 to 15 volts output. Two other improvements over contemporary design are the meter's ability to measure both current and voltage output and the double protection of the circuit: the fused primary and the automatic reset overload device for the secondary. This model has a continuous rating of 10 amperes at 5 to 8 volts and an intermittent rating of 20 amperes. Housed in a Hammertone heavy-gage steel cabinet, this instrument has rubproof control panels and may be wall-mounted or carried to the work site. Its dimensions are 10.5 × 7.75 × 8.75 inches. **15**

Soil-Suspending Starch

National Starch Products, Inc., has developed a starch product, Nu-Film, which acts as a soil-suspending agent. The new starch, an acid ester derivative containing sodium carboxylate and sulfonate groups, has demonstrated its soil-suspending ability when used with detergents. Whiteness retention tests on unsized cloth have shown that the starch prevents redeposition of dirt onto textile fibers, particularly when compounded with detergents of the alkyl aryl variety. Besides having properties useful in the formulation of laundry starches and household liquid starches, Nu-Film is suitable

for warp sizing and finishing cotton and rayon fabrics. It can be removed from fabrics without the use of enzymes. **16**

Carboy Tilter

Offered by the General Scientific Equipment Co., the new GS No. 11 carboy tilter with pouring spout assures a safe, fast, and easy method of pouring acids and other liquids from carboys. The manufacturer states that the soundly functional design of this unit saves time, effort, and materials. Long and dependable service is assured by structural steel supporting members which are either welded or riveted. The air-vent pouring spout permits smooth flow without spurts or splashes. Made of acid-resistant rubber and plastic tubing, the spout has a flow capacity of 5 gallons per minute. With air vent pouring spout, the cost of the carboy tilter is $24.95. **17**

Conductivity Cells

Armored conductivity cells for the measurement of strong sulfuric acid concentrations are announced by Industrial Instruments, Inc., manufacturers of electrolytic conductivity control equipment. The new cells are supplied in pairs, one cell serving as the reference and the other as the measuring cell. The pressure seal has been redesigned to eliminate dependence upon glass parts. Designed for pipeline installations, the cells are suitable for line pressures up to 50 pounds per square inch. The cells are made of heavy-walled borosilicate glass with platinum electrodes and matched cell constants of 1.00. Metal parts are made of either steel or Type 316 stainless steel. **18**

MANUFACTURERS' LITERATURE

Polarographic Analysis. The Electro-Chemograph, Type E, for polarographic analysis is the subject of a 16-page publication. The wide applicability of the method and instrument is outlined, while illustrations show details of design and construction. Also described is the new dropping-mercury electrode equipment designed for high mechanical stability in the performance of polarographic tests. Leeds and Northrup Co. **19**

Protection against Atomic Energy. An 8-page booklet describes protective equipment for use in connection with work on atomic energy. The publication covers respiratory protective equipment, air-sampling equipment, ventilation accessories, protective clothing, materials for contamination control, automatic artificial respiration instruments, and oxygen therapy equipment. Mine Safety Appliances Co. **20**

Laboratory Safety. Revised edition of 40-page booklet entitled "Manual of Laboratory Safety" discusses recently developed data, techniques, and equipment. Manual is a useful supplement to laboratory safety programs. Fisher Scientific Co. **21**

Lead. "Lead for Corrosion-Resistant Applications in the Chemical Process Industries" is title of well-illustrated 8-page bulletin. National Lead Co. **22**

Thermocouple Accessories. New edition of thermocouple and pyrometer accessories bulletin lists many additional items and contains new data on proper application and use of thermocouples. The 56-page bulletin contains well-illustrated catalog of hundreds of pyrometer supply items, including assembled thermocouples, thermocouple wires, extension wires, protection tubes, insulators, and accessories. Bristol Co. **23**

Kjeldahl Equipment. Booklet describes entire line of Kjeldahl apparatus which has recently been standardized. Models range from 2-unit to 24-unit digestion and distillation equipment, heated by gas or a wide choice of electrical devices. Also described are microdigestion units, as well as extraction and digestion racks. Precision Scientific Co. **24**

Water Treatment. Sodium aluminate treatment of industrial and municipal water supplies is described in a new booklet which also discusses the action of sodium aluminate as a water softener and clarifier. Pamphlet covers treatment of boiler feed water and internal boiler water treatment, in addition to the preparation of sodium aluminate solutions. Merrimac Division, Monsanto Chemical Co. **25**

New Chemicals. "Collective Volume II" is a compilation of data on several new chemicals which have become available from the company's research laboratories during the past year. Some of the chemicals covered are: β-substituted propionitriles, 3-substituted propylamines, dipropionitriles, 2-nitrodiphenylamine, 2-aminobenzenethiol, Antioxidant 2246, and sodium dicyanamide. American Cyanamid Co. **26**

Laboratory Centrifuges. Laboratory centrifuges, draining chamber inserts, and baskets are the subject of a 4-page pamphlet. One unit, the chemical model centrifuge, may be operated continuously up to the cake capacity of the basket, approximately 0.3 liter. Approximate maximum speed is 3600 r.p.m.; with porcelain basket, 2400 r.p.m. Baskets are 2.5 inches deep × 5 inches in diameter and on special order may be obtained without perforations. Eberbach & Son Co. **27**

Aerosols. A special laboratory devoted to the formulation and testing of aerosol products is described in a 4-page bulletin. In the laboratory are found the apparatus and gases needed for the manufacture of aerosol packages, a complete line of essential oils and synthetic aromatic chemicals, and mixtures that have been formulated for deodorizing and perfuming aerosols. Givaudan-Delawanna, Inc. **28**

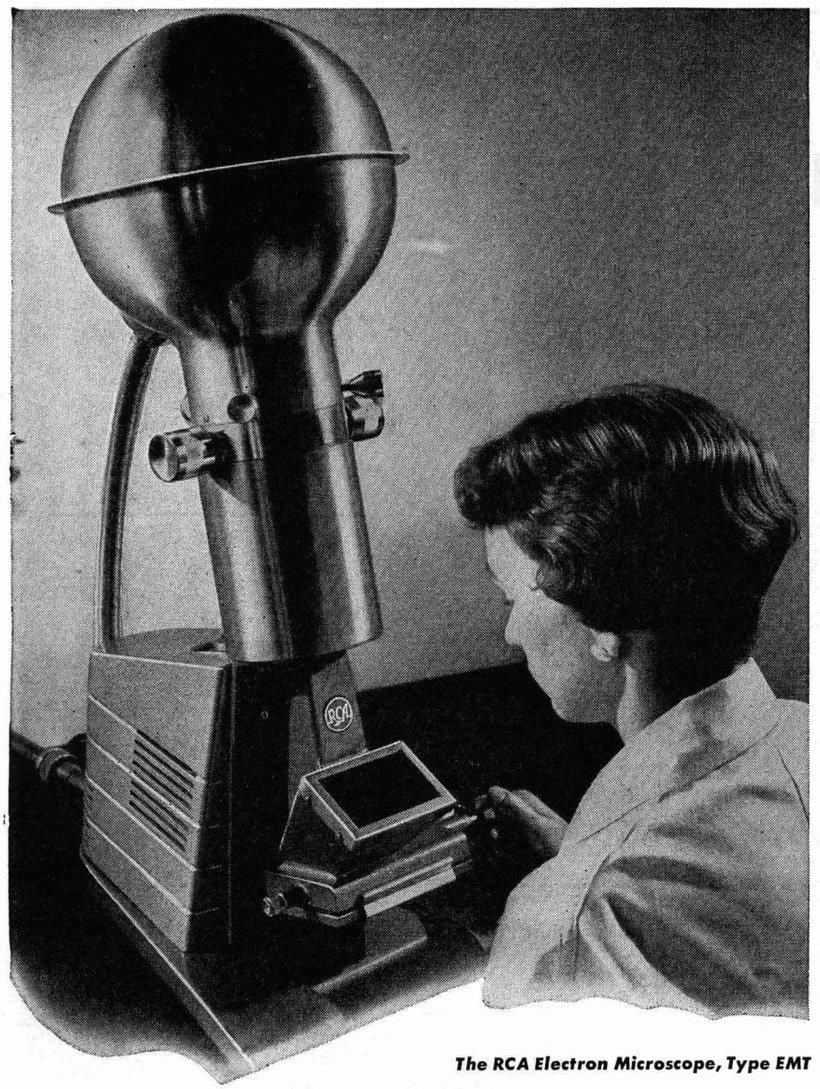
Vinyl Plastics. "Evaluation of Stabilizers for Vinyl Stocks Containing Chlorowax 40" reports the results of a laboratory study of two standard stabilizer formulations. The light and heat stability characteristics of each are summarized. Entitled "Evaluation of Inert Fillers in Vinyl Plastics," another 10-page bulletin points out the application of chemicals in plastics processing. Diamond Alkali Co. **29**

Resin Modifier. A 12-page booklet discusses use of resin in Buna N adhesives; resin is a complex mixture of phenols, phenol ethers, and polyphenols. Hercules Powder Co. **30**

Lead Stearates. Two-page bulletin describes lead stearate No. 50, a high-lead-content heat stabilizer and internal lubricant for polyvinyl chloride resins, and lead stearate No. 30, all-purpose lubricant and stabilizer. Witco Chemical Co. **31**

Density Measurement. Bulletin W-2 discusses plant production tool for continuous measurement and control of liquid density; has accuracy of ±3% of range or 0.0002 density, whichever is greater. Precision Thermometer and Instrument Co. **32**

German Drug Research. German pharmaceutical patent applications filed from October 1948 to May 1950, translated into English, are subject of Bulletin 50. Research Information Service. **33**



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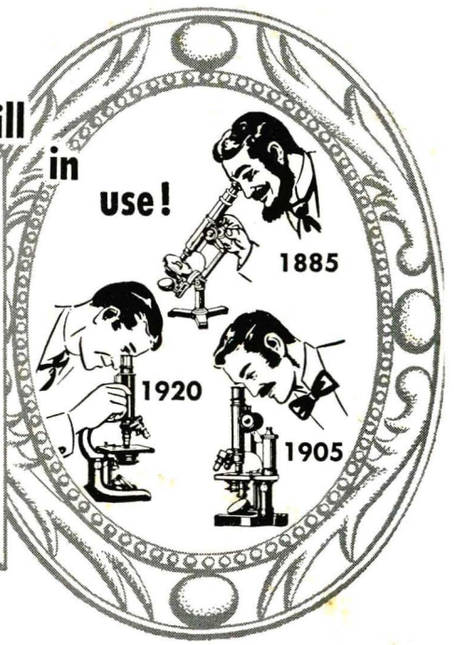

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